EPIDEMIOLOGY OF TRICHOMONIASIS IN KUMASI, GHANA

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ABSTRACT

Estimates from the WHO indicate that *Trichomonas vaginalis* (TV) infection is the most common curable sexually transmitted infection world wide. Unfortunately compared to other curable sexually transmitted infections, not much attention has been given to its control. This, however, is changing due to recent reports linking *Trichomonas vaginalis* infection to adverse birth and reproductive health outcomes and also its facilitation of acquisition of the Human immuno deficiency virus.

Studies in this thesis address aspects of the epidemiology of *Trichomonas vaginalis* infection with a view to enhancing control programmes.

Pregnant women attending antenatal clinics in Kumasi, Ghana, participated in the study. Data on socio-demographic characteristics, sexual behaviour, ano-genital hygiene, and partner behaviour were taken. Genital examinations were also done. Vaginal swabs were taken for the detection of *Trichomonas vaginalis* by 6 methods; latex agglutination, wet prep microscopy, culture, enzyme immuno-assay, polymerase chain reaction, and lateral flow. Women infected with TV were treated with standard 2g metronidazole single dose and had tests of cure done after a week.

Four main findings are highlighted;

i. socio-demographic, behavioural and clinical factors independently associated with TV infection were; young age, neither being of Akan nor northern origin, having no religious faith, and douching. Also, not using toilet roll for menstrual hygiene, complaining of a vaginal discharge, clinical detection of vaginal discharge and a vaginal pH more than 5 were independently associated with infection. However, these factors either singly or in combination, could not predict TV infection.

ii. using a combination of morphologically based traditional TV diagnostics (wet prep and culture) and molecular techniques (PCR), no evidence for the involvement of rectal and oral trichomonads in the aetiology of vaginal trichomoniasis as speculated recently, was found.

iii. a developing country-friendly TV diagnostic, the latex agglutination test, compared favourably with culture, the gold standard in TV diagnosis. With 91% sensitivity and 99% specificity, it is easy to train to use, easy to use, enables same day treatment, and does not require any equipment. It is apparently stable in field temperatures and relatively cheap.

iv. the use of 2g single dose metronidazole therapy by a directly observed strategy is feasible. It was well tolerated and had a cure rate of 99.4% after 1 week.

These findings contribute some new information to the epidemiology of TV infection. Such attributes of the latex agglutination test and the excellent clinical efficacy of single dose metronidazole therapy should make the control of TV infection feasible.

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My children have sometimes asked me who my classmates at this PhD level are. In the traditional sense of the word, I have none, I tell them. However at various times these past 3 years Dr Maria Claudia Nascimento from Brazil, Dr Edith Sanchez-

Valdez from Mexico, Dr Judith Torimiro from Cameroon (all PhD students) and I have shared office space in almost all the property of the LSHTM in the Bloomsbury area – 99 Gower street, Keppel Street, LG1 51 Bedford Square, MG3 49 Bedford square and finally M105 50 Bedford square. The humour with which we took these incessant and sometimes disruptive office space changes kept us going.

A great deal of work on trichomoniasis has been published in the grey literature by authors from Eastern Europe. I am very grateful to John Eyers of the LSHTM library for his help in organising translations of some of these works from Czech, Russian, Estonian etc into English.

Within a month of starting my PhD work, Professor David Mabey, my supervisor helped me to form my Research Advisory Panel - Dr Aura Beltran Andreasen, Dr Helen Weiss and Professor John Ackers. Their encouragement and enthusiasm for my work helped me remain focussed to the cause. A count through my diary of meetings starting 29 September 2001 to 7 September 2004, indicates I have had 43 scheduled meetings with them (not counting laboratory tuition time), individually or severally, an average of more than one meeting every month. With my highest knowledge of statistics ending at measures of central tendency before coming on this course, Dr Helen Weiss has tolerated my slow understanding of some statistical theory and managed - I hope - to steer me into understanding and using statistics correctly. Her comments on the first draft of my case control study drove me into a better application of logistic regression methods. Over the last 3 years, Professor David Mabey has been of various personalities to me – supervisor, Head of Unit, Head of Research group, co-author, mentor, teacher, advisor, travel companion, fellow consultant at WHO meetings and family friend. In all of these, the African in him has been evident. I am only disappointed that on his 4 day trip to visit me in the

field in Kumasi, there wasn't much time to savour what I have come to know is his delight – real good beer. David, please come again to Kumasi, and in the words of the late Idi Amin, I will "retaliate" your warm kindness! Kumasi Star beer (better than Gambian Julbrew) awaits you.

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Development of the study proposal and the writing of study protocols, questionnaires, and laboratory data forms, were done by me with the support of my Research Advisory Panel. I undertook and coordinated the training of field staff including interviewers, lab personnel, mid wife examiners, and data entry personnel in Ghana. With the exception of PCRs for rectal swabs which was undertaken by staff of the Microbiology lab of the Institute of Tropical Medicine, Antwerp, all other PCRs were done by me in London after appropriate training in Antwerp. Study analysis and write up of thesis was my responsibility with constructive suggestions from my Advisory panel.

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this work in good time speaks volumes for your tolerance. I hope I have not disappointed you.

Lest I inadvertently impute any imperfections in this work to all those able minds mentioned above who have assisted me, may I state that I am solely responsible for any imperfections that may be noticed in this work.

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INDEX OF ABBREVIATIONS

AP	Adhesion Proteins
BV	Bacterial vaginosis
CDC	Centres for Disease Control
CPLM	cysteine peptone liver maltose
CRU	Clinical Research Unit
Da	daltons
DFA	direct fluorescent antibody
DFID	Department for International Development
DNA	deoxyribonucleic acid
dNTPs	deoxynucleotide triphosphates
EGS	expanded gold standard
EIA	enzyme immuno-assay
ELISA	enzyme-linked immuno-sorbent assay
FW	Feinberg Whittington
HIV	human immuno deficiency virus
HPV	human papillomavirus
IgX	Immunoglobulin X
ITM	Institute for Tropical Medicine
KOH	potassium hydroxide
KATH	Komfo Anokye Teaching Hospital
KNUST	Kwame Nkrumah University of Science and Technology
LAT	latex agglutination test
LSHTM	London School of Hygiene and Tropical Medicine
NPV	negative predictive value
NAAT	nucleic acid amplification technique
OD	optical density
OR	odds ratio
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pH	hydrogen ion concentration
PPV	positive predictive value
RCT	randomised controlled trial

rRNA	ribosomal ribonucleic acid
SAVS	self administered vaginal swab
SDI	Sexually Transmitted Diseases Diagnostic Initiative
STD	sexually transmitted disease
STI	sexually transmitted infection
SMS	School of Medical Sciences
Taq	*Thermus aquaticus*
Tt	*Trichomonas tenax*
TV	*Trichomonas vaginalis*
TYM	trypticase yeast maltose
UK	United Kingdom
US	United States
VIP	Vagina Infections in Prematurity

1.0 INTRODUCTION

1.1 Background to the study

Trichomonas vaginalis (TV) is one of the commonest sexually transmitted pathogens, with an estimated annual incidence of 170 million cases worldwide [1]. It causes a wide spectrum of infections in men and women leading to great morbidity. Sequelae of infection with this flagellate include adverse pregnancy and perinatal outcomes. The Vaginal Infections in Prematurity (VIP) study in the United States (US) found that infection with *T. vaginalis* resulted in a modest increase in the risk of premature labour (relative risk 1.3) [2]. The attributable risk of *T. vaginalis* infection associated with low birth weight was 1.5% in white populations, but 11% amongst black women. The study also found that *Trichomonas vaginalis* infection in pregnancy was significantly associated with preterm delivery of a low birth weight infant (Odds ratio, 1.3).

Trichomonas vaginalis infection has also been reported as a risk factor for the transmission of Human immuno deficiency virus (HIV) infection. In a prospective study among female sex workers in Kinshasa, Laga *et al* [3] found trichomoniasis to be a risk factor for the acquisition of HIV. There have also been reports of higher concentrations of HIV in the semen of men with symptomatic trichomoniasis [4]. In HIV infected women who are concomitantly infected with *T.vaginalis*, treatment of the trichomoniasis has reduced the HIV viral load by a factor of four [5]. Strategies that incorporate treatment of trichomoniasis could be important in HIV control.

The control of any infectious disease depends on a sound understanding of its epidemiology. There are however some gaps in the epidemiology of *Trichomonas vaginalis* infection, which data could be important for its effective control. Important amongst these is whether other human trichomonads apart from *T.vaginalis* are

involved in the aetiology of urogenital trichomoniasis. Conventional wisdom dictates that these other trichomonads cannot colonise the vagina. This follows from earlier studies which reported that all human trichomonads are site specific and that one could not find the other human trichomonads i.e. *Pentatrichomonas hominis* and *Trichomonas tenax* (which are found in the large intestine and oral cavity respectively), in the urogenital tract. Most of these studies utilised diagnostic methods relying on the morphology of the organism. In recent times, it has been reported that morphological differentiation of human trichomonads is difficult [6] as they all look morphologically alike at microscopy. Also, trichomonads have been reported from non traditional body sites [7,8,9] using mostly more sensitive molecular methods, thus questioning the postulates that they are site specific.

The finding in a community survey of 40% adolescent girls in Zambia with vaginal trichomoniasis (based on morphology) who deny ever having had sexual intercourse [10], and a report finding *P.hominis* in vaginal swabs (by polymerase chain reaction) from a similar population [11] leads to speculations of whether the trichomonads found in the adolescents in Zambia are those supposedly not transmitted sexually, but getting into the vagina from a rectal reservoir (the traditional site of *Pentatrichomonas hominis*). The control of *T.vaginalis* infection is based on measures against sexual transmission. Involvement of other trichomonads (not transmitted sexually) in the aetiology, would have a bearing on measures which help control the spread and sequelae of infection.

Modern molecular diagnostic methods should make a study of which trichomonad species is found in women diagnosed with *Trichomonas vaginalis* possible.

In analysing a model of treatment interventions for *T.vaginalis*, Bowden and Garnet[12] demonstrated that screening populations for infection and treating those found

infected would have a far greater impact on reducing rates of infection than the present syndromic management approaches used in many developing country settings which have the highest burden of infection. Such screening programmes would however depend on the availability of diagnostic tests not only with good test performances, but also tests which can be done with less technologically demanding equipment, performed with less skilled labour, at low cost, and under conditions as found in developing country settings. The tests would also have to be rapid to enable same day treatment. Some presently available tests could fulfil this purpose.

Laboratory tests presently available for the detection of *T.vaginalis* are either insensitive or relatively expensive for use in resource poor countries. Finding socio-demographic, biologic, sexual, genital hygiene and clinical factors which could predict infection would be useful in selecting subjects for further investigation and treatment of infection.

Work presented in this thesis investigates the above problems and hopefully should help our understanding of the aetiology of vaginal trichomoniasis in relation to other human trichomonads. The choice of laboratory diagnostic tests for effective screening and control of disease would also be aided by work presented.

1.2 Purpose, Aims and Objectives of Study

The purpose of the study is to improve the understanding of the epidemiology of *Trichomonas vaginalis* and other related human trichomonads in the aetiology of vaginal trichomoniasis and evaluate laboratory diagnostic tests with a view to improving the control of infection.

1.2.1 Aims of study: to study the epidemiology of trichomoniasis in Kumasi, Ghana.

1.2.2 Primary objective: ascertain whether human trichomonads other than *T.vaginalis* are involved in the aetiology of urogenital trichomoniasis.

1.2.3 Secondary objectives:

i. investigate factors related to sociodemographic, biologic, ano-genital hygiene practices, sexual practices, and partners' sexual habits that are associated with vaginal trichomoniasis in pregnant women.
ii. ascertain clinical correlates of *T.vaginalis* infection in pregnant women
iii. compare different laboratory diagnostic methods (latex agglutination, wet prep examination, culture, enzyme immunoassay, lateral flow and polymerase chain reaction) for *T. vaginalis* infection.
iv. investigate the involvement of human trichomonads other than *Trichomonas vaginalis* in the aetiology of vaginal trichomoniasis.
v. determine the species of trichomonads found in the vagina, rectum and oral cavity of study participants.
vi. determine the clinical efficacy of single 2g dose metronidazole therapy in vaginal trichomoniasis in Ghana.

1.3 Outline of work to be presented

Relevant published literature relating to the biology of *T.vaginalis*, its pathogenicity, epidemiology and clinical disease in men and women, the probable role of extra-genital trichomonads in the aetiology of genital trichomoniasis is presented in the first part of chapter 2. The second part of the chapter presents literature on the laboratory diagnosis of *T.vaginalis* infection and treatment issues. Methods for all aspects of the study are described in chapter 3. Chapter 4 details results and

discussion of socio-demographic, behavioural, biologic, sexual practices, genital hygiene factors associated with infection. Results and discussion of Clinical correlates of *T.vaginalis* infection are written up in Chapter 5, and a combination of both the socio-demographic etc factors and clinical correlates of *T.vaginalis* infection written up in chapter 6. Write up of the results and discussion of Comparison of *T.vaginalis* diagnostic methods is presented in Chapter 7. Chapter 8 presents results and discussions relating to the involvement of human trichomonads other than *T.vaginalis* in the aetiology of vaginal trichomoniasis. Work on species of trichomonads found in the vagina, rectum and mouth is presented in Chapter 9. Chapter 10 is on the results and discussion of the Clinical efficacy of 2g single dose metronidazole in the treatment of vaginal trichomoniasis. Study conclusions, with suggestions for further research are found in Chapter 11.

1.4 Collaborating Institutions and Ethical Clearance

Collaborators for this study comprise the School of Medical Sciences of the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, The Komfo Anokye Teaching Hospital, Kumasi, Ghana, the Ghana Health Service, the Clinical Research Unit of the London School of Hygiene and Tropical Medicine, and the Institute of Tropical Medicine, Antwerp.

Ethical and Research clearance for the study was obtained from both the Committee on Human Research, Ethics and Publications of the School of Medical Sciences and The Research Ethics Committee of the London School of Hygiene and Tropical Medicine.

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2.0 LITERATURE REVIEW

Search strategy

A search of English language literature in electronic databases including Pub Med, Web of Knowledge, Embase, BIDS, Africa Heathline and the WHO Reproductive Health Library was conducted, using the key words *Trichomonas vaginalis* and trichomoniasis. Access to personal hard copy libraries of my Research Advisory Panel was granted and searched, and the abstracts of recent meetings of the International Society for STD Research were also scrutinised.

2.1 *Trichomonas vaginalis* and Trichomoniasis

2.1.1 The organism

2.1.1.1 History

Trichomonas vaginalis is a protozoon parasite found in the urogenital tract. The organism has been implicated in many genitourinary conditions, commonly including vaginitis in women, and non gonococcal urethritis in men. It is one of the commonest sexually transmitted pathogens worldwide, with an estimated annual incidence of 170 million cases worldwide [1]. In 1975, *T. vaginalis* was estimated to have caused approximately 3-4 million infections in the US and over 1.5 million in Great Britain [13]. The epidemiology of the disease is still not well understood and some practitioners continue to question its importance. There is growing evidence however of its importance in its morbid role in conditions such as vaginitis and non gonococcal urethritis and its likely role in facilitating the transmission of HIV.

T. vaginalis was first described by Donné in a preparation of fresh vaginal exudates in 1836 [14]. It was long regarded as a harmless inhabitant of the vagina [15,16]. When Kunstler[16] found it in the female urinary tract in 1883 and Marchand [16] also from the

male urinary tract in 1884, its designation as harmless still did not change. In 1916, Hoehne [17] demonstrated a relationship between *T.vaginalis* and symptomatic vaginal discharge. It was not until 24 years later that Trussel and Plass [18] fulfilled Koch's postulates by transmitting the disease to volunteers by the intravaginal inoculation of axenic cultures of the organism. This was confirmed 2 years later by Hesseltine and colleagues [19].

Even with publication of reports of many cases of this organism, clinicians did not give it much significance as they were more interested in the diagnosis and treatment of gonorrhoea and syphilis. However, by 1950, the significance of sexually transmitted infections other than gonorrhoea and syphilis had become apparent [20], and investigators began to view *T. vaginalis* as important [21]. Yet still today, not much importance has been given to it compared to *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. On today's market are commercial kits for the diagnosis of chlamydia and gonorrhoea based on nucleic acid amplification technology (Polymerase chain reaction). There is none for *T. vaginalis*, the diagnosis of which is still based on the same 1836 wet preparation method of Donné [14]. At many conferences on sexually transmitted infections, delegates fill sessions where new diagnostics for *Chlamydia trachomatis* and *N. gonorrhoeae* are showcased, while only a handful are seen at sessions for *T. vaginalis* diagnostics. One rapid diagnostic test for *T. vaginalis*, the Kalon TV latex test [22] with a purported sensitivity and specificity of 95 and 97% respectively, has, since evaluations in the UK in 1988, not seen much routine use and discussion of it. Why would people want to invest in a test for a disease which to them is so uncommon and associated with minimal morbidity and sequelae?

2.1.1.2 Taxonomy and biology

Together with other human trichomonads described later, *T.vaginalis* is a member of the zoomastigophorean order of the phylum Sarcomastigophora and belongs to the protozoan order Trichomonadida. The order contains more than 100 species most of which are commensals found in the intestinal tract of mammals and birds. Although a eukaryote, *T.vaginalis* has properties similar to bacteria; it lacks mitochondria, 28s ribosomes and regulated glycolysis. Like higher eukaryotes, it possesses DNA sequences and peptides related to cell division.

The organism, which is flagellated, varies in size and shape, with an average length of 10μm and width of 7μm [23]. The appearance tends to be more uniform in axenic cultures i.e, pear shaped or oval, and it takes an amoeboid shape when attached to vaginal epithelial cells [24].

The protozoan possesses 5 flagellae, four of which are located at its anterior portion. The fifth is incorporated within the undulating membrane of the parasite. The flagellae and the undulating membrane give the parasite a characteristic quivering motility [23]. The cytoskeleton is composed of tubulin and actin fibres. The nucleus of the parasite is located at its anterior end, and as in other eukaryotes, is surrounded by a porous nuclear membrane. A slender hyaline rod-like structure, the axostyle, runs from the nucleus and bisects the protozoan horizontally. It protrudes the posterior end and terminates in a sharp point. The axostyle is thought to anchor the parasite to vaginal epithelial cells.

Glycogen granules can be observed in living cells under light microscopy. These are catalase negative and produce hydrogenosomes, which are important in metabolism. The hydrogenosomes generate hydrogen which combines with oxygen to form water,

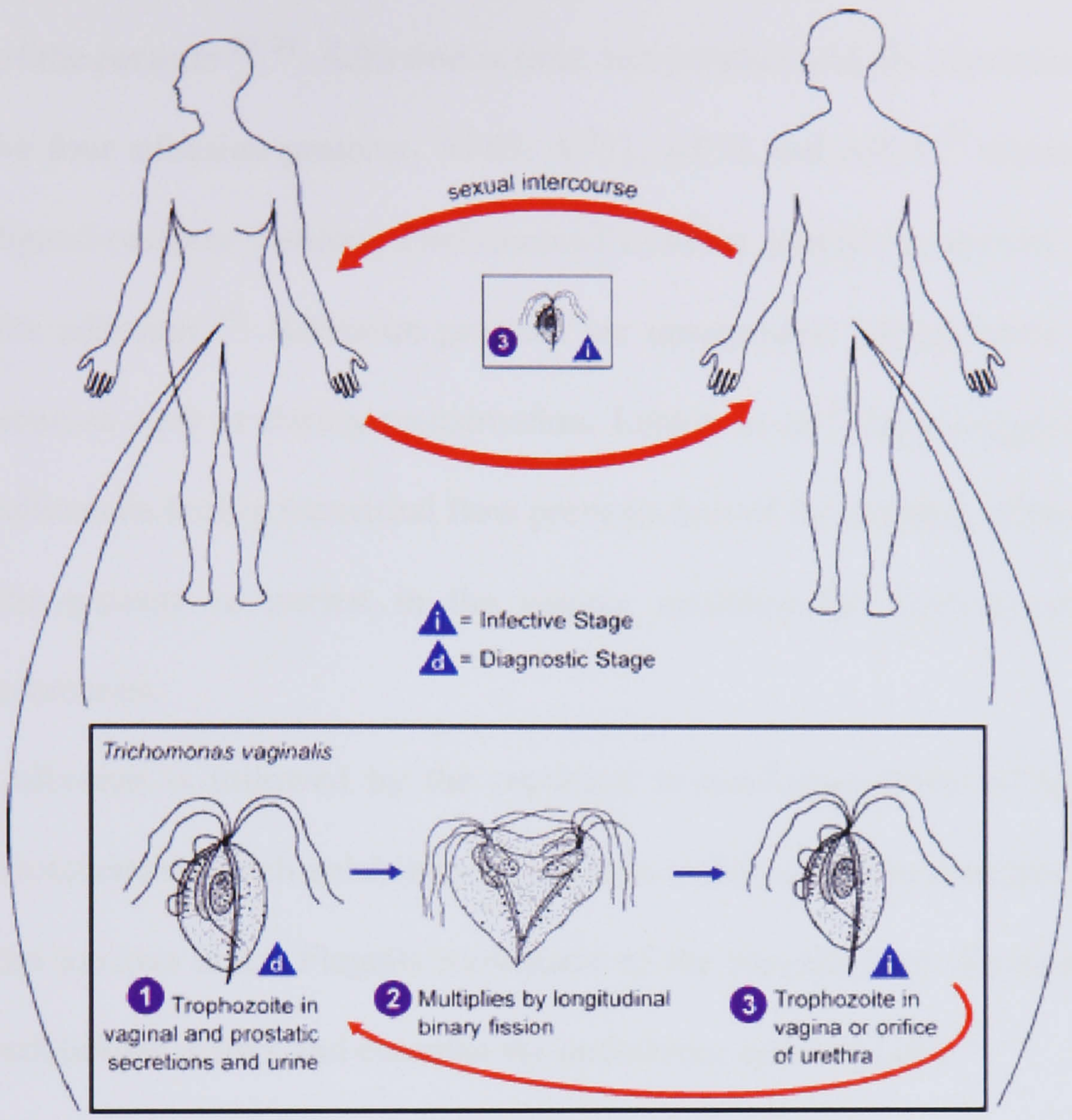
thus removing oxygen from the vaginal ecosystem and creating an anaerobic environment in which *T.vaginalis* and other anaerobes thrive.

In keeping with its anaerobic status, *T.vaginalis* derives its carbon from the reduction of glucose and glycogen into succinate, acetate, malate and hydrogen. It also produces some carbon dioxide, but not through the Krebs' cycle.

2.1.1.3 Life cycle

T.vaginalis reproduces by mitotic division and longitudinal fission which occurs every 8-12 hours in optimal conditions. The parasite exists only in trophozoite form, lacking a cystic stage [25]. Its life cycle is poorly understood. The parasite lives on the epithelium of the uro-genital tract to which it is anchored by its axostyle. Infection is usually transmitted by sexual intercourse and continual re-infection of a sexual partner by an untreated partner is common.

Figure 2.1 Life cycle of T.vaginalis



http://www.tigr.org (Accessed 12 September 2004)

2.1.1.4 Pathogenicity

The cell surface of *T.vaginalis* plays a major role in adhesion, host-parasite interaction and nutrient acquisition. Proteins and glycoproteins displayed on its surface are used for these functions.

The mucous layer of the genital tract is the first host surface encountered by trichomonads. Mucins, large glycoproteins with sizes between 1-35 x 10^6 Da, are the major proteins found in mucus [26]. Its size, gel-like property and heavy glycosylation make mucin a formidable barrier to pathogens colonising the underlying epithelium. The outcome of the interaction between trichomonad and the host mucous membrane determines the success or otherwise of *T. vaginalis* colonising a host.

Adhesion of trichomonads to the vaginal epithelial cells is critical in the pathogenesis of the parasite [27, 28]. Adhesion is time, temperature and pH dependent and is mediated by four adhesion proteins, AP65, AP51, AP33 and AP23 [29] which act in a specific ligand-receptor fashion. Trichomonad cysteine proteinase activity is also necessary for adhesion [30]. Adhesion proteins are upregulated during times of abundant iron sources such as during menstruation. Lehker *et al* [31] have suggested that increased adherence during menstrual flow prevents loss of the parasite. Adherence thus allows the parasite to persist in the vagina, avoiding the flushing action of mucosal secretions.

Adhesion is followed by the secretion of mucinases (most of which are cysteine proteinases) which solubilise the mucous matrix and then detaches the parasite from the mucous layer. Flagella movement of the parasite then allows it to penetrate the solubilised matrix and colonise the underlying epithelial cell [32].

Thus, adherence, proteinase activity and motility are the major mechanisms necessary for *T.vaginalis* to traverse the vagina mucosa and establish pathogenicity.

2.1.1.5 Immune system evasion

Ability of the parasite to evade the immune system is important for its survival. Avoidance of complement is the main strategy used by *T.vaginalis* to overcome the human immune system. *T.vaginalis* activates the alternative complement pathway [33]. The organism takes advantage of a habitat with very little complement activity such as cervico-vaginal mucus [34]. Menstrual blood is the main source of complement available to the vagina. Even then, the complement activity of menstrual blood is only about half that of venous blood, and about a third of menstrual blood samples have no complement activity at all [34]. Again, iron contributes to complement

resistance in *T.vaginalis* as resistance to complement is dependent upon a high concentration of iron [34], a nutrient abundant in menstrual secretions. Iron seems to upregulate the expression of cysteine proteinases, which have been found to degrade the C3 portion of complement on the surface of the organism, allowing it to evade complement mediated destruction[34].

Another means of immune system evasion is the possession of all *T.vaginalis* isolates of the high molecular weight immunogen, P230 on their surface. This immunogen undergoes conformational changes (epitope phenotypic variation) that allows evasion[35].

Other mechanisms of immune evasion by the trichomonad include degradation of IgG, IgM, and IgA by cysteine proteinases secreted by the parasite [36], the secretion of large amounts of soluble antigens which may neutralise antibody or cytotoxic T lymphocytes, thus short-circuiting specific anti-T.vaginalis defence mechanisms [37], and the parasite coating itself with host plasma proteins, a form of immunological mimicry [38].

2.1.1.6 Transmission.

Evidence from epidemiological and human inoculation studies indicate that urogenital trichomoniasis is transmitted almost exclusively by sexual intercourse. There is a high prevalence rate in male sexual partners of infected women[39, 40], and vice versa[40, 41]. In such cases recurrent trichomonas vaginitis has been cured only after the parasites had been eradicated from the genital tract of the sexual partner. The infection has also been seen more frequently in patients attending sexually transmitted diseases (STD) clinics and in commercial sex workers[42, 43]. Trichomoniasis is also often associated with other STDs [44, 45]. *T.vaginalis* has also

been isolated almost exclusively from urogenital sites i.e. vagina, cervix, bladder, urethra and Bartholin and Skene's glands in women and the anterior urethra, external genitalia, prostate, epididymis and semen of men.

Human inoculation studies to fulfil Koch's postulates were carried out between 1940-1955. Intravaginal [19] and intraurethral [46] inoculations led to infection of 75% of women and five of five men respectively and none of controls. However, like other sexually transmitted agents e.g. gonococcus and herpes simplex 2, that can survive outside the human host, there has been speculation about non sexual transmission especially in prepubertal girls. Charles [47] reported 27% of girls below 12 years with leucorrhoea having trichomonas vaginitis. He speculated transmission through communal use of water tanks and ponds. Adu-Sarkodie [48] also reported infection in a mother and 3 daughters, speculating transmission through joint family use of bathing sponge and towel. Recently in a study looking at reasons for the differential spread of HIV in different African sub regions, Buvé [10] and colleagues reported 40% of adolescent girls in Ndola, Zambia to be infected with *T.vaginalis*. These girls denied ever having had sexual intercourse and the authors could not explain this finding by under-reporting of sexual behaviour by the girls. They suggested that this infection could be due to intestinal trichomonads (*P.hominis*), that these faecal trichomonads could have contaminated the self administered vaginal swabs or that intestinal trichomonads do really colonise the vagina via the perineum. Even though the authors dismiss under-reporting of sexual behaviour by these girls, it could be so in reality. In many African societies, non consensual sex as in rape or sexual defilement especially if perpetrated by a family member may be counted as no sex by the teenagers, to correspond to perceived social norms. Also, sight should not be lost of problems in the reporting of sexual behaviour in adolescent surveys. There is

evidence that the type of question asked and the mode of survey administration (face to face interviews, paper and pencil self administered interviews or computer assisted self interviews) significantly affect the responses of adolescents to sensitive questions [49].

Non sexual transmission of *T.vaginalis* by contaminated douche nozzles, specula and toilet seats has been speculated [40].

Between 2-17% of female infants born to infected mothers have developed urogenital trichomoniasis at birth [13]. In the presence of maternal oestrogens, the neonatal vaginal epithelium resembles that of the adult and is therefore susceptible to *T.vaginalis* infection. When the neonate's vaginal epithelium assumes a prepubertal state after maternal oestrogens have been metabolised within 3-4 *weeks* of birth, it becomes relatively resistant to *T.vaginalis* infection and there is resolution of infection and discharge [13].

Though there has been no cogent proof of non sexual transmission, the theoretical possibility exists.

2.2 Epidemiology of *Trichomonas vaginalis* infection

2.2.1 Introduction

Trichomonas vaginalis (TV) infection has been encountered world wide, in every climate and with no seasonal variability. It has a cosmopolitan distribution and has been identified in all racial groups and socioeconomic strata.

Humans are the only natural hosts for *T.vaginalis*. To perpetuate itself, *T.vaginalis* depends on the host for survival. It has no cyst phase and is susceptible to desiccation [50] and high temperatures, but can survive for long periods outside conditions of high humidity. *T vaginalis* has been isolated from swimming pools, baths, and poorly

chlorinated water [51, 52, 53]. The organism has survived in vaginal exudates at 10^0C for up to 48 hours [54], in voided urine for as long as 3 hours, in semen ejaculates for 6 hours [55] and in 35^0C water in wet washcloths for up to 24 hours [20, 39]. About one third of contaminated washcloths yielded viable organisms after 2 or 3 hours and 10% even after 24 hours [20]. Viable organisms have also been isolated in vaginal exudates that have been allowed to dry for 3 – 6 hours [56, 57]. Whittington has suggested that moisture is essential for the survival of the organism [54]. Viability of *T.vaginalis* has been preserved in Stuart's transport medium at room temperature for up to 3 days and at refrigerator temperature for 9 days [40].

T.vaginalis has been found to contaminate toilet seats [40, 58]. In a unique experiment, Whittington [40] found that 11 of 30 (37%) infected women left urine on toilet seats after use and that 4 (36%) of these samples yielded viable trichomonads. After seeding toilet seats with trichomonas-laden vaginal exudates, Whittington again found that the organisms survived from less than 10 minutes to up to 45 minutes [40].

2.2.2 Is trichomoniasis uncommon?

As far back as 1947, Trussel estimated that between 20-25% of the female population in the US were infected with *T.vaginalis* [59]. Before that, a study of women attending a US Naval hospital in 1938 showed that 24.6% had *T. vaginalis* [60], and 14% of male recruits to the US Army in 1943 were also infected [61]. A US vaginal cytology survey in the 1950s showed infection in 60.9% of black women and 8.1% of white women [39]. An investigation of Pap smears of 38,000 "healthy women" in the US in the 1960s demonstrated infection in 30.4% black women and 10.7% white women [62]. These studies, predating the introduction of metronidazole to the US in

1959 and the early 1960s, show unequivocally, the high prevalence of *Trichomonas vaginalis* infection in that society then.

The situation improved with the introduction of metronidazole. However the disparity in risk between ethnic groups and people with different socio-economic standing has continued into the post metronidazole era. While infection was seen in 0.5% of antenatal clinic attenders in an Australian urban setting [63], it was seen in 25% indigenous women in rural northern Australia, [64], and 45% Melanesean women in Papua New Guinea [65]. While 0.7% of European men with a urethral discharge [66, 67] had trichomonas urethritis in the 1990s, the parasite was reported from 10% of Melanesean men with urethritis [65]. Differential prevalence among black and white women in America still remains, with infection rates of 22.8% compared to 6.1% respectively [2]. Prevalences of 20-65% in women in various population groups have been reported from Africa [10, 68, 69, 70, 71, 72, 73].

Even though these prevalence data cannot be directly compared due in part to different diagnostic methods and inherent biases in all studies, they nevertheless show unequivocally that *Trichomonas vaginalis* infection is common especially in economically disadvantaged populations in both developed and developing countries.

2.2.3 Epidemiology in women.

Most studies on the distribution of trichomonas vaginitis are biased by the lack of random sampling and have a bias towards populations likely to be diseased (e.g. STD clinic attenders) or a convenience population such as antenatal clinic attenders. Studies also vary in the sensitivity and specificity of testing procedures employed. Infection rates of 0.5% - 65% [2, 63, 64, 65, 73] have been reported from antenatal clinics, with the highest prevalence coming from poor under resourced communities.

High risk sexual behaviour i.e. high rates of sexual partner change and/or sex in exchange for money increases the risk of female acquisition of *T.vaginalis* infection[69, 74, 75], as does low socioeconomic status [76]. In Cotonou, women who exchange sex for money have significantly higher prevalence of *T.vaginalis* infection than those who do not (18.2% vs. 3%) [10].

Trichomoniasis has also been associated with the presence of other sexually transmitted infections (STI) [69, 77], the likely explanation being confounding by sexual behaviour. In Yaoundé, Cameroon, infection was significantly associated with gonorrhoea (p=0.02) while in Ndola, Zambia infection was associated with Chlamydia infection (p=0.02).[10]

A high prevalence in multiparous women, women married at an early age, and pregnant women has also been reported [10, 78].

Being from a black ethnic background has also been consistently associated with *T. vaginalis* acquisition in the US [2], with black/white case ratios as high as 8:1 reported. Some commentators attribute this to confounding by poor socioeconomic circumstances.

In most studies, the prevalence of trichomoniasis seems to increase with increasing age, [10, 79] with peak rates occurring in women in their thirties and forties [20, 39]. This may not suggest that older women are at any higher risk of disease than younger ones. It may indicate a disease with a relatively long duration, prone to chronicity, and not cured by inadvertent antibiotic use.

It has been postulated that the use of oral contraceptives is associated with a decreased risk of trichomoniasis. In a study of 23,756 patients, the relative risk of trichomoniasis for oral contraceptive users compared to non users was 0.49 (95%CI 0.46-0.53) [80], suggesting that non user women are twice more likely to acquire

T.vaginalis infection than users of oral contraception. This effect has not been seen with gonorrhoea. The biologic nature of this is difficult to explain. It may be due to confounding by socio-economic standing, in that low contraceptive use is prevalent in communities of poor socio-economic standing in whom high *T. vaginalis* prevalence is also found. Lossick suggests that high levels of oestrogen are not conducive to *T.vaginalis* infection [81]. This view however contrasts the explanation of transient colonization of infants born to *T.vaginalis* infected women, that this transient state is due to maternally transferred oestrogens to the infant, and that colonisation is lost after degradation of the hormone in the infant [13].

2.2.4 Epidemiology in men.

Just like trichomonal vaginitis in women, prevalence studies in men suffer from biases of patient selection and diagnostic methods. Trichomonas infections are also not routinely diagnosed in men because of the apparent limited ability of traditional tests ie wet prep and culture to detect the organism in the male genital tract. Prevalences of 3-18% have been reported in various populations of men around the world [82, 83, 84, 85]. The highest reported prevalence has been 68% in men in Chile [86], and the lowest, 1% among men in Seattle [87].

In a population based study of men aged 15-54 years from Tanzania, Watson-Jones *et al* reported a prevalence of 11% using direct microscopy and culture [88]. Using a combination of culture and the polymerase chain reaction (PCR), Hobbs and colleagues reported a prevalence of 17% in men attending a sexually transmitted diseases clinic in Malawi [4]. In determining the aetiologic role of pathogens other than *N.gonorrhoeae* and *C. trachomatis* in urethral discharges among men from West

Africa, Pépin *et al* found *T.vaginalis* in 13.4% of subjects as against 13.8% and 10% of *N.gonorrhoeae* and *C.trachomatis* respectively [89].

Krieger [90], studying 447 men at risk of sexually transmitted diseases, found a prevalence of 6% for *T.vaginalis* infection. In these men, risk factors significantly associated with infection were sexual exposure to a woman with trichomoniasis (odds ratio 3.7, 95%CI 1.9-7.4), and a history of previous treatment for trichomoniasis or urethritis (odds ratio 3.1, 95%CI 1.4-6.9).

The importance of *T.vaginalis* as a cause of genitourinary syndromes in men has been the source of controversy. Investigators interested in sexually transmitted diseases in women, especially in the US, believe that asymptomatic transmission is the rule for men infected with *T.vaginalis* [87]. In contrast, European investigators have found that *T. vaginalis* infection in men is a major cause of morbidity [91]. Clinical conditions in men that have been associated with trichomoniasis include non gonococcal urethritis, prostatitis, balanoposthitis, epididymitis and infertility. The strongest epidemiologic association is between *T.vaginalis* and non gonococcal urethritis.

Though studies have demonstrated an aetiological link between *T.vaginalis* and non gonococcal urethritis, the precise proportion of cases of non gonococcal urethritis attributable to trichomoniasis has not been established. This may vary in different populations and also with different diagnostic tests. Whilst Bakare *et al* found *T.vaginalis* in 18 out of 215 men (8%) with non gonococcal urethritis in Nigeria using wet prep and culture [92], Schwebke *et al* found infection in 19.9% of American men with non gonococcal urethritis, using PCR [93].

2.2.5 Partner Infection studies

Rates of partner infection in men are different from that in women. While most female sexual partners of infected men are infected with trichomoniasis (67-100%) [40, 90], investigators have reported only 14-60% [39, 84, 90, 94, 95] infection of male partners of women with trichomoniasis. The low infection rate in men may be due to transmission inefficiency from female to male, poor sensitivities of current TV diagnostics in men, or spontaneous resolution of infection in the male [94]. Some authors have ascribed spontaneous resolution in men to be the result of the constant washing action of the urethra by urine and the anti-trichominicidal property of zinc found in prostatic secretions [96].

In summary, the epidemiology of trichomoniasis is characteristic of a sexually transmitted infection. The infective dose of the organism in women appears small, and the incidence rate is high. Asymptomatic disease is common in both sexes and almost the rule in men. The infection also resolves spontaneously in some men. Trichomoniasis shares many of the risk factors of the other sexually transmitted infections, but also differs in other respects.

2.3 Clinical features of *T. vaginalis* infection

2.3.1 Is *T. vaginalis* infection clinically trivial?

The symptoms of trichomonas vaginitis are troubling. Before the era of metronidazole, women were subjected to prolonged and often unpleasant treatment courses, some of which were quite toxic [97]. Their willingness to undergo these treatments suggests the degree of discomfort associated with infection. Again, because most of the treatments at that time were topical and administered by physicians, most of whom were men, women must have suffered the indignity of

exposing themselves for such treatments, which they did because they needed relief from the distress of the vaginitis.

Keighley, reminiscing over the huge success of oral metronidazole in the treatment of female trichomoniasis after 10 years of use, said, "it is good to pause and contemplate the change that oral medication for trichomonal vaginitis has made in women's lives. Flagyl is now taken as a matter of course and a whole generation has no knowledge of the suffering of women with trichomoniasis before its introduction – the indignities and discomfort of the perpetual local treatments, douches, paintings, insufflations and insertion of pessaries. All these things women suffered for months and sometimes years on end, only to relapse when the treatment was discontinued [98]".

2.3.2 Clinical presentation and disease in women.

Although many studies have looked at the epidemiology and clinical disease of human urogenital trichomoniasis, most of these concern various aspects of the disease in women.[20, 99, 100]. Possible explanations can be deduced. Trichomoniasis in women is associated with well described clinical syndromes, considerable morbidity, and standard diagnostic algorithms [101, 102, 103]. In contrast, trichomoniasis in men is associated with poorly delineated clinical syndromes, uncertain morbidity and there is no familiar algorithm with respect to its diagnosis. The presentation of trichomoniasis in females is considered in many clinical textbooks, unlike that of men. It is not surprising that medical disciplines outside the practice of specialist Genitourinary medicine and Urology do not consider male trichomoniasis a clinical entity.

Trichomonas vaginalis principally infects squamous epithelium in the genital tract [104]. In the adult the ectocervix is much more affected and disease is rarely reported

from the endocervix. Trichomonads have been recovered from the urethra and Skene's glands in 90% of infected women [20, 40]. Infection at these sites may cause dysuria or discharge from the urethra or Skene's ducts.

The spectrum of infection ranges from the asymptomatic carrier state to florid vaginitis. Factors responsible for this are not clear but could include differences in intrinsic virulence of individual strains and individual host susceptibility. Clinical evidence for the latter includes the observation that even among infected female sexual partners of an individual man, the spectrum of disease ranges from mild to severe. Since these epidemiologically linked women are most likely infected with the same strain of trichomonad, differences in clinical expression is probably related to host factors.

About 9-56% [20, 50, 101, 105, 110] of trichomonal infections in women are asymptomatic in various population groups. 75% women in Zimbabwe denied any symptoms on questioning, out of which 16% had trichomoniasis [106].

A third of asymptomatic women have been observed to become symptomatic within 6 months [107]. However, one cannot discount re-infection or the probable co-infection with a more virulent strain after initial infection with a less virulent strain. A number of studies have reported exacerbation of trichomoniasis symptoms during or immediately after a menstrual period [20, 50, 108]. This could be due to the presence of iron in menstrual blood. Iron upregulates the virulence of *T.vaginalis* [31].

The incubation period for trichomonal vaginitis has been estimated to range between 3-28 days [20, 50, 109]. Vaginal discharge and vulvo-vaginal irritation are the most common complaints. Discharge is described by 50-75% of symptomatic women [101, 107, 110]. A study in 200 symptomatic Nigerian women demonstrated 74% had *T.vaginalis* infection [70]. The discharge is perceived as malodorous by only about

10% of infected women [111, 112]. Vulvovaginal irritation which can be severe enough to awaken the patient at night is noted in a quarter to a fifth of women [101, 110, 113, 114]. Up to a fifth of women report some degree of dyspareunia [110], and lower abdominal pain by only 5-12% [65, 113]. Dysuria is described by about 30% of patients [115, 116, 117]. There is no evidence that trichomoniasis is more symptomatic in pregnancy or among users of oral contraceptives [66, 115, 121].

Pastorek and colleagues [118] studied over 13,000 pregnant women in the US and reported that clinical findings significantly associated with *T.vaginalis* infection were a yellow, green or bloody vaginal discharge, abnormal odour, a vaginal pH>5, increased amount of discharge, and abnormal consistency of the discharge. However the clinical usefulness of these features either alone or in combination were minimal in the prediction of infection, not having sufficient sensitivity and specificity. In Cameroon, a clinician's ability to predict vaginal infection with *T.vaginalis* based solely on clinical grounds was shown to have a positive predictive value of 42% [119]. Serbian researchers have also reported poor correlation between clinical features and diagnosis of infection [120].

Cervical pathology in the form of colpitis macularis or strawberry cervix results from microscopic punctate haemorrhages of the cervix. *T.vaginalis* has been significantly associated with this clinical finding [118].

2.3.3 Clinical presentation and disease in men.

Clinical presentation of trichomoniasis in men has been described in many studies. These studies have been conducted in sexual partners of infected women and men presenting genitourinary tract complaints. The majority of sexual contacts of infected women are asymptomatic [20, 84, 112]. Among 23 East African men with trichomoniasis,

19 were asymptomatic [126]. Jirovic and Petru comment that "in the male, infection is mostly latent or persists in subclinical form [20]".

The incubation period of trichomonal urethritis has been reported severally to be between 3-8 days [94, 127]. Much wider variability however, has been reported by Czech researchers, according to whom this varies from 1 day to 3 months [6, 127].

Clinical symptomatology of infected men varies widely. Weston and Nicol [94] evaluated 93 men with trichomoniasis – 52% had obvious urethral discharge, 26% complained of dysuria, and 5% noted increased urinary frequency, while 21% had no symptoms. Wisdom and Dunlop [128] described 92 men with symptomatic trichomoniasis. Urethral discharge was found commonly in 64%, urethral irritation in 62%, urethral pain in 24%, increased urinary frequency in 5%, lower abdominal pain in 4% and balanoposthitis in 3%. Only 7% of the 92 cases had evidence of prostatitis. Catterall [129] described clinical findings in 108 symptomatic men out of 126 with trichomoniasis. 83% complained of urethral discharge, 26 (24%) noted urethral irritation, 14% had dysuria, 4 (4%) experienced increased urinary frequency, 3% noted haematuria, and 1% had epididymitis. Clearly in all these cases urethritis was the predominant syndrome. Kuberski, in contrast, found that majority of his patients (77% of 26 men) with trichomoniasis had clinical evidence of prostatitis [86].

2.3.3.1 Spontaneous resolution of male urethral trichomoniasis

Spontaneous resolution of male trichomonal urethritis has been reported by several investigators, with the most convincing work coming from Weston and Nicol [94] (Table 2.1). They investigated the prevalence of trichomoniasis among male sexual partners of infected women as a function of the interval between last sexual exposure and examination. When the interval between examination and exposure was 2 days,

T.vaginalis was found in 70% of patients. After 2 weeks, infection could be identified in only 30%. Beyond this period, it became difficult to evaluate the presence of *T.vaginalis* as the numbers of patients examined became small. Other workers suggest however that spontaneous resolution of untreated *T.vaginalis* in men is highly unusual. In this wise, Coutts *et al* [130] stated that "chronic infection of the male genitourinary tract is very common". Watt and Jennison [84] found that 13 of 15 men with *T.vaginalis* were still infected after 2 weeks, but these men were apparently not instructed to refrain from sexual intercourse with their infected partners. In an accident of history, Catterall [129] treated 12 men who had urethral trichomoniasis with oxytetracycline (known to be totally ineffective today) for 5 days. Follow up after 3 months showed that 10 patients (83%) still harboured the protozoa. Similar findings have been reported by Whittington [40] and other Eastern European workers [20].

Not many studies have utilised semen as source material for TV diagnosis in men. The above studies utilised urine and/or urethral swab samples from the men. In a recent study using semen as diagnostic material, 75% male sexual partners of infected women have been reported to be concurrently infected. This may indicate the superiority of the use of seminal fluid in the diagnosis of TV infection in men [344]. The long term consequences of untreated trichomoniasis in men are not known.

Table 2.1. Prevalence of urogenital trichomoniasis among male sexual partners of infected women (data from Weston and Nicol [94])

Interval between last sexual exposure and evaluation (days)	*T.vaginalis* identified/contacts examined	Prevalence (%)
2	16/23	70
4	16/29	55
6	7/27	26
8	10/25	40
10	8/21	38
14	9/30	30
20	8/15	53
30	3/13	23
40	1 /4	-
50	2/2	-
60	1/ 4	-
60+	7/8	-
Unknown	7/8	-
	Total 93/207	Mean 45

2.4 Sequelae of infection with *T. vaginalis.*

Compared to infection with *N.gonorrhoeae* and *C.trachomatis*, many clinicians have considered infection with *T.vaginalis* to be without sequelae and less of a public health problem, but merely a nuisance to the sufferers. However recent data on the association of *Trichomonas vaginalis* and premature labour and low birth weight and its facilitation of HIV transmission may question this.

The Vaginal Infections in Prematurity (VIP) study in the US found that infection with *T. vaginalis* was associated with a modest increase in the risk of premature labour (relative risk 1.3) [2]. Though this may be of minor importance in populations at low risk of *T.vaginalis* infection, the attributable risk could be substantial in endemic populations, such as Africa. In the VIP study cited, the attributable risk of *T. vaginalis* infection associated with low birth weight was 1.5% in white populations, but 11% amongst black women. The VIP study [2] also found that *Trichomonas vaginalis* infection in pregnancy was significantly associated with preterm delivery

of a low birth weight infant (Odds ratio, 1.3). Unfortunately recent trials have found that the treatment of *T.vaginalis* infection in pregnancy does not improve pregnancy outcome and may be harmful [131, 132, 133]. In one of the studies [131], as much as 8g metronidazole (recommended dose 2g) was given to the women. Probable toxicity from this could be responsible for the excess adverse pregnancy events in the women who took the drug.

In addition to its role in adverse pregnancy outcomes, *T. vaginalis* has been associated with endometritis, infertility, ectopic pregnancy, and non human papillomavirus (HPV) associated cervical neoplasia [134 135, 136, 137]. It has also been linked to respiratory disease among infants delivered vaginally to infected mothers [138].

In men, an association between *T.vaginalis* and epididymitis, prostatitis, and infertility has been reported [21, 139, 140].

Most of the studies on the HIV /STI co-factor link have looked at genitoulcerative conditions and bacterial inflammation STI such as, gonorrhoea and chlamydia infection. Only a few studies have specifically looked at *T.vaginalis* and HIV. These have shown an association between trichomoniasis and HIV infection, suggesting a two to three fold increase in HIV transmission [3, 141, 142]. A cross sectional study conducted among 1209 female sex workers in Cote d'Ivoire found an association between HIV and trichomonas infection in bivariate analysis (crude odds ratio 1.8, 95% CI 1.3-2.7) [141]. In another cross sectional study in Tanzania, among 359 women admitted to a hospital for gynaecological complaints, TV was more common in women with HIV infection in multivariate analysis (odds ratio 2.96, 95% CI not provided, $p<0.001$). While these cross sectional studies are limited by temporal ambiguity, i.e. lack on information on whether TV infection preceded HIV, one

prospective study by Laga *et al*, reinforced such findings. In this study in which 431 HIV negative female sex workers in Zaire were evaluated over time, prior TV infection was associated with almost a 2 fold increased rate of HIV seroconversion in multivariate analysis even though this was of borderline significance [3]. Sorvillo and Kerndt [143, 144] have pointed out that even a modest increase will translate into an attributable fraction for HIV of nearly 20% in areas where the prevalence of *T. vaginalis* infection is 25%. The prevalence of gonorrhoea and chlamydia infection in many African countries is low compared to trichomoniasis. In the Rakai study of mass STD treatment to reduce HIV incidence, the prevalence of gonorrhoea and chlamydia infection was 2.1% and 4.0% respectively in a subgroup of the study population [145]. In the Mwanza survey, the prevalences were 2.2% and 0.7% respectively [146]. The attributable fraction of HIV cases caused by *N.gonorrhoeae* and *C.trachomatis* in these populations will therefore be small, even though the relative risk of transmission to the individual may be substantial. *T vaginalis* prevalence is however consistently higher on the continent with reports of 24% in Uganda. It is thus possible that the control of *T. vaginalis* could be effective in reducing the incidence of HIV.

Hobbs *et al* [4] report finding a 6 fold concentration of HIV in the semen of men symptomatic for *T. vaginalis* infection. They did not find this in men asymptomatic for *T. vaginalis* infection or men who were symptomatic for other conditions of unknown aetiology. This may mean men with symptomatic trichomonas infection are more infectious for the transmission of HIV. Wang and colleagues [5] noticed a 4.2 fold reduction in the vaginal HIV-1 load after treating *T.vaginalis* infected women who also concomitantly were HIV infected. These findings suggest that HIV-1

intervention strategies that incorporate the treatment of *T. vaginalis* infection could be important in the control of HIV infection.

The adverse pregnancy events, negative reproductive health events and increased susceptibility to HIV infection as shown by the studies above indicate the seriousness of the sequelae of *T. vaginalis* infection, and thus necessitate as much attention and action as given to infections due to *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

2.5 Extragenital Trichomonads

2.5.1 Human Trichomonads

There are 3 species of the family Trichomonadidae that are found in humans – *Trichomonas vaginalis, Pentatrichomonas hominis, and Trichomonas tenax.*[147]. All 3 species can be found outside the genitourinary tract.

2.5.1.1 *Trichomonas vaginalis*

In human clinical studies, most cases of findings of *T. vaginalis* extra genitally have involved contact between the genitalia and the mouth. Rebhun in 1964 [148] reported a case of pulmonary trichomoniasis in a man presumably infected after oral sex with his infected female partner. The flagellate was identified based on the case history and its morphologic characteristics on microscopy and not on its cultivation. Trezhalmy [149] and Brenner and Simon [150] also reported finding trichomonads which they said were *T. vaginalis* based on morphology, in foul smelling purulent lesions from the mouth and tongue scrapings of patients with sore throat, dysphagia and severe glossitis. These patients recovered on metronidazole therapy.

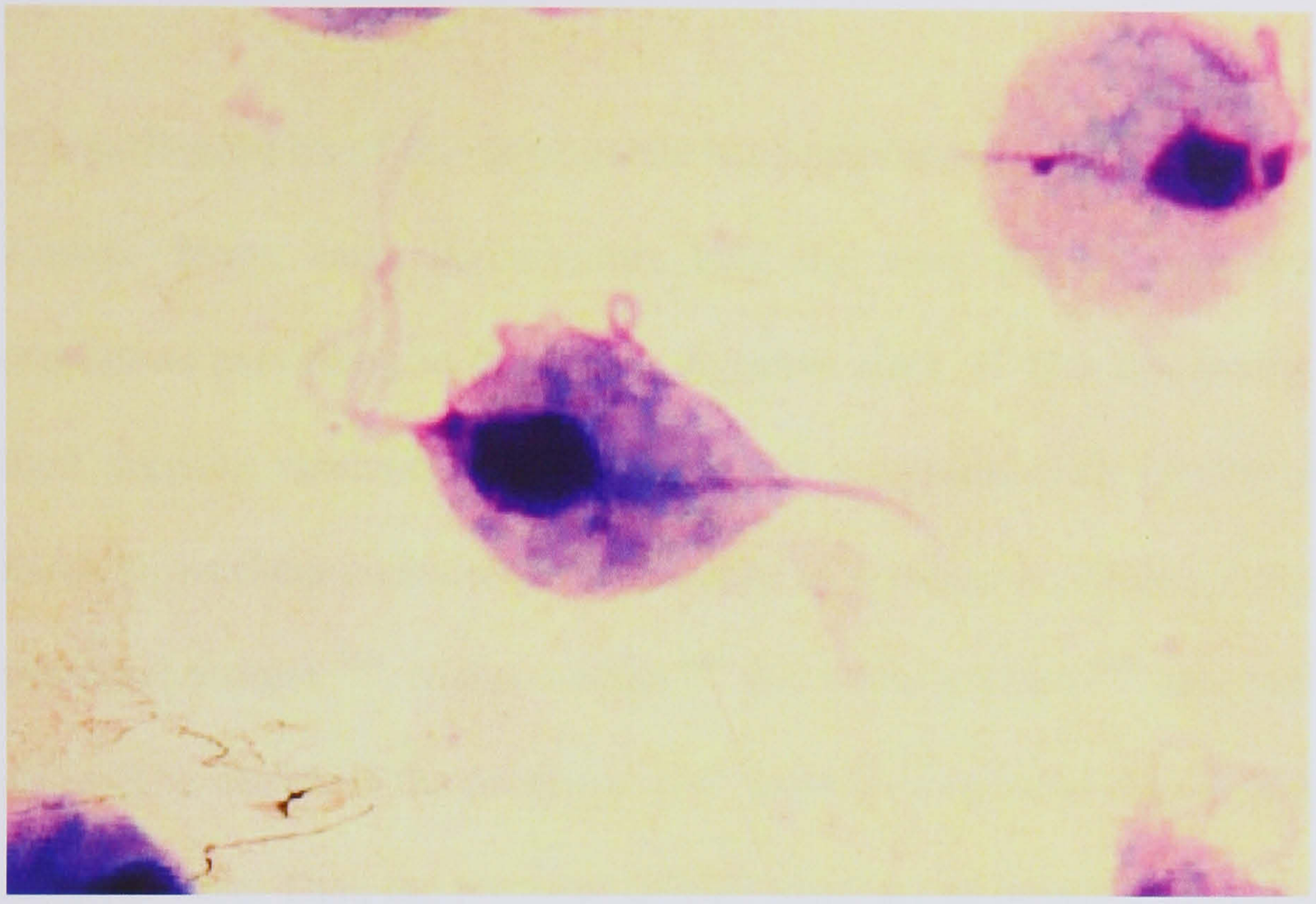
There have also been documented cases of bronchitis, pneumonia and rhinitis presumably caused by *T.vaginalis* in new born babies [138, 151]. Sources of these trichomonads have been traced to infected mothers with vaginal trichomoniasis [152]. Colonisation of neonates with *T.vaginalis* from infected mothers has been reported. The babies are known to rid themselves of these trichomonads when the level of maternal hormones transferred to them during pregnancy wanes [152].

Most of these findings as mentioned above have been based on direct microscopic findings of *T. vaginalis* and sometimes by culture. However, there needs to be caution in delineating these isolates as *T.vaginalis* just because there has been contact in a way with the genitalia. Differentiating between individual trichomonads by wet film examination is difficult as all trichomonads morphologically look alike under the microscope[6]. It is not improbable that other species could be mistaken to be *Trichomonas vaginalis* and reported as such. Indeed Hersh [6] writing about *T. tenax* in 1985 indicated, "because of their morphological resemblance to other trichomonads and their marked protoplasmic plasticity and the environmental and genetic variation in organism size and shape, identification of flagellate species using only the wet preparation is unreliable"

In recent times, using nucleic acid amplification techniques, *T. vaginalis* has been recovered from the pharynx of 3 men. Only one of them was symptomatic for pharyngitis. In another, *T. vaginalis* was additionally identified from his genital secretions. All three men admitted to oro-genital contact with their female partners. The *T. vaginalis* infection status of their partners was not reported and TV strain typing not done [8]. Another study using genomic amplification and sequencing of the small-subunit rRNA of *T.*vaginalis reported finding *T.vaginalis* together with

Pneumocystis sp in bronchoalveolar lavage of an adult African patient in France [153] with respiratory disease. No history of oro-genital contact was indicated.

Figure 2.2 Giemsa stain, *T.vaginalis*



www.medicine.cmu.ac.th (Accessed 12 September 2004)

These reports of findings of *T. vaginalis* apparently causing disease in sites other than genitourinary, especially using molecular techniques suggest that *T.vaginalis* can establish itself in mucosae other than the genitourinary mucosa.

2.5.1.2 *Pentatrichomonas hominis*

This can be found in the large intestine of man. However, flagellates structurally indistinguishable from this species have been reported from the caecum of non human primates, cats, dogs and a variety of rodents [154, 155].

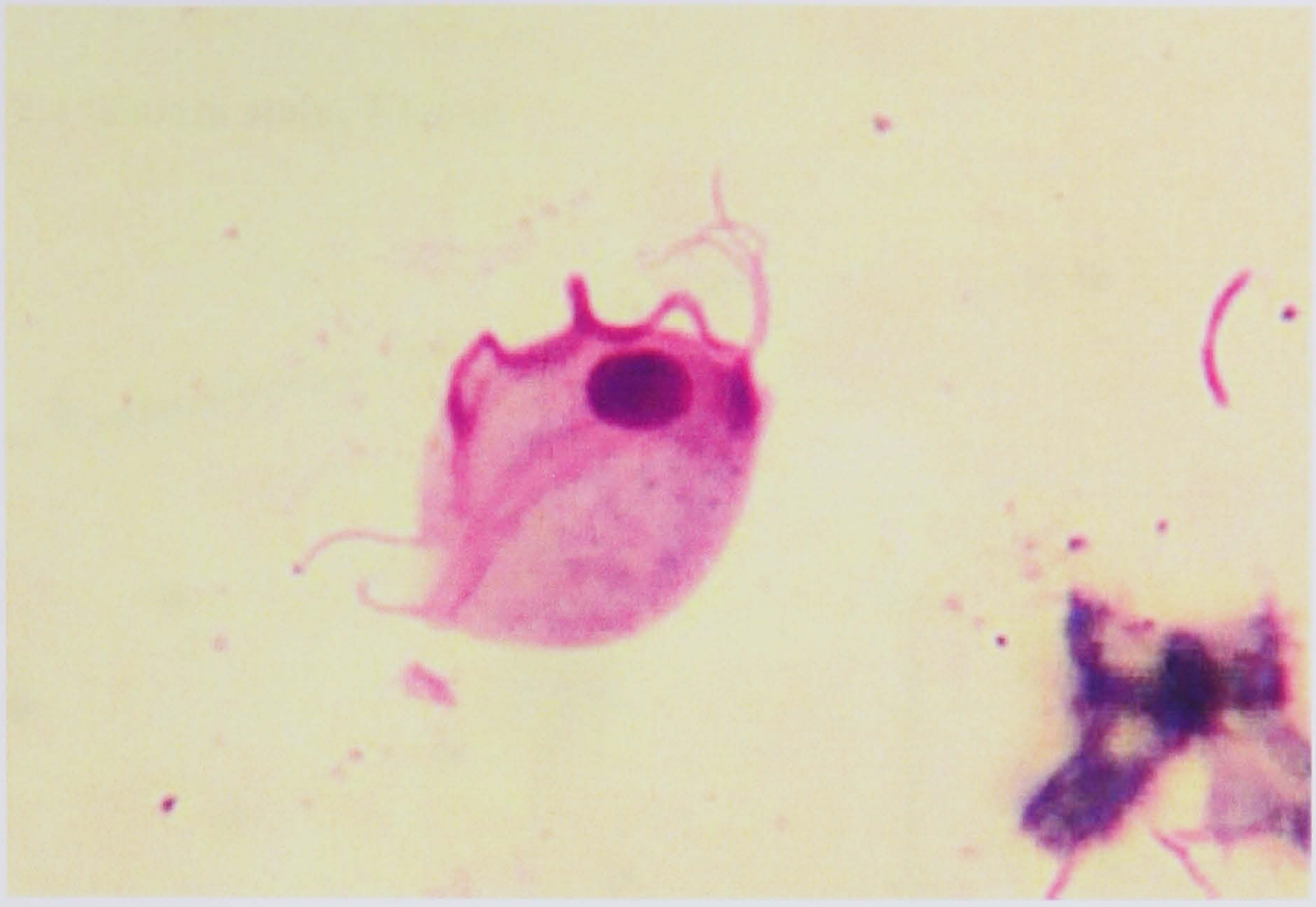
Morphologically, it is pear shaped, 15-18um long, 14-15um wide. Its undulating membrane is about half the length of its body. As with *T.vaginalis* and *T.tenax*, it has a single nucleus and the axostyle conforms to that of the trichomonidadae.

P.hominis is transmitted faeco-orally and is more prevalent in tropical and subtropical environments than temperate regions. The warm and humid environment and unhygienic conditions in the tropics favour the transmission of this protozoon [156, 157].

The pathogenicity of this trophozoite has been the subject of controversy, like that of *T.tenax*. Many early investigators held the view that at least under favourable conditions prevailing in the host's digestive tract, *P. hominis* strains could express their inherent virulence, causing intestinal symptoms with varying severity [158, 159]. Estonian workers considered this parasite as pathogenic, finding it in many cases of intestinal disease [160]. Manson-Bahr [161] and other workers [162] asserted that it is often difficult to decide if the presence of *P. hominis* in stool is the cause or consequence of the disease. That, the presence of high numbers of flagellates might be due in large part to their swarming in an abnormal liquid environment, as for example in the case of bacillary dysentery. Though Manson-Bahr has often been cited as opposed to the pathogenicity of *P. hominis*, he does concede that inflammatory states associated with a variety of intestinal disturbances resulting in the secretion of mucus by goblet cells provide excellent culture medium for the trichomonads. Others have supported that although in some instances serious prolonged intestinal disturbance is due to *P. hominis*, this parasite would cause little damage in a generally healthy host [163].

Response to metronidazole therapy leading to the elimination of symptoms and manifestation of disease has also been used by the proponents of the inherent pathogenicity of *P. hominis* [164]. It is difficult however, to accept as proof of pathogenicity, metronidazole elimination of trichomonads in a milieu inhabited by mixed organisms many of whom are susceptible to metronidazole (e.g. anaerobic bacteria).

Figure 2.3 Giemsa stain, *P.hominis*



www.medicine.cmu.ac.th (Accessed 12 September 2004)

Outside the gut, *P. hominis* has been reported from diseased states of the respiratory tract of humans [9, 165]. Apart from one report where identification was by nucleic acid amplification techniques [9], others were based on morphologic, antigenic and physiologic characteristics, insufficient for differentiation between the 3 human trichomonad species. A liver abscess caused by *P.hominis* has also been described [166]. A case of mixed bacterial meningitis with *P.hominis* subsequent to a fistula from the gastro-intestinal tract has also been described [167].

Recently, Crucitti *et al* have developed a sensitive and specific PCR for the detection of *P.hominis* in biological samples [11]. Using this assay, they identified 2 cases of *P.hominis* in vaginal swabs from Mwanza, Tanzania.

These extra-intestinal reports of *P. hominis* suggest it too, could establish in mucosae other than intestinal.

Trichomonas tenax

Figure 2.4 Giemsa stain, *T.tenax*

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T.tenax is a parasite of the oral cavity [156]. In humans, *T. tenax* is found in the periodontum and adjacent areas including alveolar gingivae, gingival pockets, maxillary sinuses and paranasal sinuses, especially in people with poor oral hygiene. Morphologically, it is pear shaped, has a single nucleus, and its undulating membrane runs the entire length of the organism. Its axostyle is typical of all trichomonads. Distinguishing *T.tenax* from other human trichomonads can be difficult as underpinned by Hersh [6].

Opinions about the pathogenicity of *T.tenax* vary widely. While some investigators consider it a true pathogen, others say it is an oral commensal. Yet others believe this oral flagellate is an opportunistic saprophyte, favoured by congenital and acquired diseases of the oral cavity. Proponents of its pathogenicity argue that even though *T.tenax* has been found in the oral cavity of people with evidently healthy mouths,

the prevalence is higher in those with diseases of the oral cavity. Jaskoski found *T.tenax* prevalence at 66% in people with poor oral conditions [168] in the US. This has been corroborated in other studies [169, 170, 171]. These workers strengthen their stand with the good recovery from disease after metronidazole therapy [172, 173]. However, Blake [174], and Abramova and Voskresenskaia [175] in their contribution on this issue suggested that the interactions between oral protozoa, bacteria and yeasts in the aetiology of oral disease which is often polymicrobial, was important in the pathogenicity of the disease, stating that an oral environment altered by the metabolic products of these organisms could cause the appearance of pathologic states by a hitherto commensal. Hence it is not the inherent pathogenicity of *T.tenax* producing the periodontal diseases, but a breakdown of oral defences by the combined metabolic products of protozoa, yeasts and bacteria that enabled *T.tenax* to act as an opportunistic pathogen. Their reasoning concurs with the thinking on the pathophysiology of bacterial vaginosis, another polymicrobial disease state [176]. Again, the metronidazole therapeutic response, although plausible, should be taken with care, as metronidazole, apart from its anti-protozoal activity, has a wide action spectrum against anaerobic bacteria incriminated in disease of the oral cavity.

Significant numbers of *T.tenax* were recovered from lung aspirates in 2 studies from Russia where it was found in 10-17% of patients with lung disease [177]. Other cases of respiratory infection with *T.tenax* have been described [6, 178], thus underpinning the fact that this oral trichomonad can establish itself outside the oral mucosa. There have been no reports of *T.tenax* colonisation of the genitourinary tract. Could it be seeded into the genitalia during oro-genital contact?

Molecular based techniques using primers specific for the 3 trichomonads, could be helpful in elucidating species of trichomonads found in the vagina, mouth and gut.

2.6 Trichomonads and Immunodefficiency

The possible link between trichomonads and immunodeficiency remains unclear. Several cases involving different trichomonad species have been reported. This includes peritonitis in an immunocompromised patient, oesophagitis in a man with AIDS, and lymph node trichomoniasis in an elderly woman with refractory anaemia [166]. *T.vaginalis* has recently been shown to be associated with pelvic inflammatory disease in women infected with HIV [179], noted in the pharynxes of HIV infected men with histories of oro-genital activity [8], and in the bronchoalveolar lavage of an AIDS patient [153]. Again, trichomoniasis has been implicated in the acquisition and transmission of HIV infection, and the prevalence of vaginal trichomoniasis is higher in HIV positive women [3]. In the 4 city study looking at the differential prevalences of HIV in Africa, trichomoniasis was associated with high HIV prevalent countries.[10] Although cause and effect are not clearly delineated, immunosuppression per se is not believed to facilitate trichomonads infection [180], and trichomoniasis is not yet recognised as an opportunistic disease.

2.7 Could extra genital trichomonads (i.e. *Trichomonas tenax* and *Pentatrichomonas hominis*) be involved in the aetiology of urogenital trichomoniasis?

Honigberg [147] indicates that there is overwhelming data based on morphologic, immunologic and physiologic evidence that distinct species of trichomonads are limited to the oral cavity, large intestines and urogenital tract of man. Also, cross infection studies done by Westphal and others, suggest that these 3 species are unable to establish lasting infections in any part of the body other than the ones in which they are traditionally found [156, 181]. However work done by later investigators

show that morphological differentiation between the 3 species is difficult [6], and while *P.hominis* and *T.vaginalis* need different media for optimum growth, both do grow on conventional media [182]. Wenyon [183], one of the respected authorities on parasitic protozoa was not convinced that there existed in humans 3 different trichomonad species because they all looked and behaved similarly in culture media. This lack of clear differentiation between the trichomonads is commented on in Walton and Bacharach's review of the literature when reporting on 3 cases of pulmonary trichomoniasis [178]. Commenting on this diagnostic dilemma, they state, "no reliable identification is available from the earlier investigators. Half of these reports assign the organism (*T.tenax*) to the genus *Trichomonas*, and half of these presume it to be the same species which inhabits the intestines of man".

The difficulty in differentiating between trichomonads by morphology has also been highlighted in veterinary medical practice. Romatowski [184] initially reported isolating *P.hominis* from the gut of kittens. Later, Levy *et al* [185], reported that these flagellates had been misdiagnosed, that rRNA gene sequencing had identified the isolates as *Tritrichomonas foetus*. The practice of culling bulls with positive preputial cultures for the sexually transmitted pathogen *Tritrichomonas foetus* due to economic reasons (causes infertility in herds of virgin bulls), has been questioned in recent studies. Until recently, the diagnosis of *T.foetus* preputial infection had been by microscopic examination and culture of preputial scrapings and/or washings. Using a combination of culture, *T.foetus* specific primers PCR assay and pan-trichomonad primers PCR assay, workers have observed the existence of easily mis-identified non-*T.foetus* trichomonads (most of which originate from the gut) in the bovine prepuce. They recommend that culture of preputial scrapings or washings should be augmented with specific PCR assays in confirming infection [186, 187].

It is clear from both human and veterinary medical practice, that differentiating individual trichomonad species on morphology and physiologic criteria may not be easy as stated by earlier investigators.

Even though vaginal trichomoniasis has traditionally been thought to be caused by *T. vaginalis*, some recent reports suggest the probable involvement of other trichomonads. Buvé [10] and colleagues report finding *T. vaginalis* in 40% of adolescent girls who denied ever having sexual intercourse. They (Buvé *et al*) thought this finding could not be explained by under-reporting of the sexual behaviour of the girls. Could it be that in situations of poor ano-genital hygiene or certain genital hygiene practices (wiping forward after defecation i.e. cleaning from the anus towards the vagina, douching, etc) gut related *P. hominis* is transmitted into the vagina and reported as T. *vaginalis* at microscopy due to similar microscopic morphology? Could some TV strains have their origin in the rectum? These are not novel suggestions. As far back as the 1940s, some investigators regarded the intestine as the source of infection in the vagina, believing that intestinal trichomonads could become adapted to the vagina [188, 191]. Also, explaining why vaginal trichomoniasis was twice more common in black than white women in the US in 1947, Trussell attributed this to poor anogenital hygiene practices in the black women [100].

The plausibility of finding *P.hominis* in the vagina has been highlighted by a recent report detecting 2 cases in vaginal swabs using nucleic acid amplification techniques [11]. The clinical significance of this finding is not known. It is not improbable that faecal contamination of the perineum picked up by the self administered vaginal swabs is responsible. Also, transient colonisation of the vagina by *P.hominis* cannot be ruled out. That the vagina may be contaminated with faecal matter is not unusual.

Hegner [189] and Dobell [190] claimed to have successfully introduced *P.hominis* into the vagina of monkeys for 20 days and 3 years respectively. Karnaky in 1934 claimed to have established *P.hominis* in human vagina for 4-5 days [191]. This assertion of Karnaky was ridiculed by his peers when in a subsequent declaration, he said the intestinally implanted *P.hominis* had later changed into *T.vaginalis*! Importantly, this underpins the difficulties in making a morphological difference in diagnosis of these trichomonads.

Could poor ano-genital hygiene or the practice of oro-vaginal sex or rectal intercourse followed by vaginal penetration introduce oral or gut related trichomonads into the vagina? The practice of oral sex has been speculated as the possible mode of transmission of *T. vaginalis* found in the pharynx of men admitting oro-vaginal contact with their partners. [8].

Lurie *et al* [192] postulate that short ano-vaginal distances could influence conditions that promote microbial colonisation of the vagina. This was after they had isolated significantly more gut related microbes (including *Trichomonad sp* – they failed to mention which species) from the vagina of women with recurrent vaginitis than controls. Probable seeding of organisms from the rectum into the vagina is recognised in the practice of Genito-urinary medicine where chronic or recurrent Candida infection of the vagina is thought by some practitioners to come from a rectal reservoir of the yeast [126, 193]. Could this happen for *P.hominis* which later is misdiagnosed as T.*vaginalis* on morphological criteria?

Some investigators working with *T. vaginalis* have reported observing different sized and shaped trichomonads from genital secretions. As to whether these are different strains of *T. vaginalis* or different trichomonad species thought to be *T.vaginalis* is not known [194].

With modern nucleic acid amplification techniques using specific primers for the 3 human trichomonad species, it is possible to study this phenomenon.

2.8 Laboratory Diagnosis of *Trichomonas vaginalis*

2.8.1 Introduction

Due to the sometimes asymptomatic nature of urogenital trichomoniasis in both men and women, and the poor predictive value of symptoms and signs for infection, laboratory detection of the protozoon is important. This chapter reviews laboratory tests available for the diagnosis of *Trichomonas vaginalis* infection.

2.8.2 Wet mount microscopy

The most cost effective method for the laboratory diagnosis of *T. vaginalis* is the wet mount microscopy described by Donné in 1836 [14]. In this, technique, also called the saline wet mount, vaginal or cervical secretions are mixed with 0.9% saline and observed under the microscope where the characteristic motility and morphology of the protozoon is seen. This technique has a variable sensitivity, with reports ranging from 38% - 82% [195, 196, 197]. The wide variation in sensitivity may be due to different culture media used as reference tests in the studies. *T.vaginalis* culture media are known to have different sensitivities [198].

The low sensitivity has been ascribed to the loss of motility by the organisms after they have been taken away from body temperature [21]. Delays between specimen collection and microscopic examination reduce viability of the organism. Kingston and colleagues reported that 20% of wet mount positive samples became negative within 10 minutes of the initial reading, increasing to 35% and 78% by 30 minutes and 2 hours respectively [199]. Also, the abundance of epithelial cells and

polymorphonuclear leucocytes present in the vaginal or cervical exudates can obscure the visualisation of the protozoon, particularly movement of its flagella. Low numbers of protozoa in the exudates as may occur after douching [101] can also reduce the sensitivity of wet mount microscopy.

Interpretation of the smear can be limited if the microscopist is inexperienced or scans the field too quickly. This notwithstanding, the saline wet mount has a high specificity and is the accepted routine procedure in many clinics for clinic based diagnosis in many settings. Specificities ranging from 75% to 100% have been reported, with most ranging from 95 -100% [101,195, 200]. At least 44% of Genitourinary Medicine clinics in the UK[201] and many in the developing world diagnose infection by this method alone.

2.8.3 Culture

Successful axenic cultivation of *Trichomonas vaginalis* in the 1940s [202, 203] paved the way for the development of culture media to grow *T.vaginalis.* Presently, this is judged to be the most sensitive method for the diagnosis of *T.vaginalis*. It is the reference "gold standard" test. Low numbers of organisms present in exudates can grow and be identified [204]. A swab of the sample is immersed in the culture broth and incubated at 37°C. The culture is observed daily for the characteristic motility of the trichomonad. If this is not seen usually by day 7 of cultivation, the culture is declared negative.

Different culture media for *T.vaginalis* exist which allow for axenic cultivation of the parasite. Basically the media consist of buffered salt solutions, protein hydrolysates, liver digests or yeast extracts, reducing agents and serum. Axenic cultivation, which eliminates bacteria and fungal contaminants that inevitably accompany *T.vaginalis*

when transferred from a patient source, is achieved by the addition of antibiotics and antifungals to the culture medium. Antibiotics such as penicillin, streptomycin, gentamicin, neomycin and chloramphenicol and antifungls as nystatin, amphotericin and miconazole have been used in axenisation.

Examples of *T.vaginalis* culture media are Feinberg Whittington (FW), Stenton, Simplified trypticase medium (STS), Cysteine peptone liver maltose (CPLM), Diamond's (also known as TYM- trypticase yeast maltose), Hollanders, Kupferberg Trichosel, Oxoid, Lumsden, Lowe's and various modifications of these media. Media may either be liquid (eg. Feinberg Whittington, Lumsden and Oxoid), or semi liquid by the addition of 0.05%-0.1% agar (eg CPLM, TYM or Lowe's). The agar reduces the diffusion of oxygen into the medium and allows better growth. In general, *Trichomonas vaginalis* grows better when incubated under partial or complete anaerobic conditions.

Sensitivities of these media vary, with reports of 71-100% [198]. Cox and Nicoll [205] found in a comparative study that Feinberg Whittington and Bushby media were more sensitive than Oxoid and Stenton Media. Rayner [206] detected over two times the number of *T.vaginalis* infections when using CPLM than FW. In recent times, Diamond's modified medium has been shown to be the most sensitive medium for the culture of *T. vaginalis* [207, 208.]

Culture methods are more sensitive than wet prep microscopy, especially in situations where there are low numbers of organisms. However in situations where there are non-viable organisms in exudates, culture cannot detect them. Other limitations to the use of culture are that it takes 2-7 days to identify the trichomonads, by which time infected patients may continue to transmit the infection.

Personnel costs, daily examination, and limited availability to clinicians are other limitations to its use.

In order to improve the acceptability of culture and reduce time spent in the preparation of media, a plastic envelope system (PES), acting as one self-contained system and permitting both immediate microscopic examination and culture was devised [209]. This has been reported to give equivalent results to traditional culture methods discussed above, but significantly more sensitive than wet prep methods. Its long shelf life (at least 1 year) and contents of dry medium in tablet form which are reconstituted just before use, are additional advantages [209].

Another system, the InPouch system, is a two-chambered bag allowing one to perform immediate wet mount microscopy through the bag, as well as culture without daily sampling. In a comparison of the performance of the InPouch against other culture media, Draper *et al* [210] reported that it performed as well as Diamond's modified medium, InPouch with a sensitivity of 88.25% and Diamond's with a sensitivity of 91.12%. Levi *et al* reported similarly [211]. In a study in 2 sexually transmitted diseases clinics, the InPouch detected 15 more patients with *T.vaginalis* infection (all of whom were wet prep negative) as against 12 by Hollander's culture medium. The sensitivity of Hollander's medium compared to InPouch was thus 80% [212]. In all these studies, culture of specimens in the various media did not detect all positive specimens i.e. *T.vaginalis* grew in some media and not in others. The reason for this differential growth is believed to be *Trichomonas vaginalis* strain variation due probably to differences in nutritional requirements [210].

The InPouch system offers a lot of advantages over other *T.vaginalis* traditional culture systems. Once the specimen is placed in the chamber, microscopic visualisation can be done directly through the bag and daily sampling, which may

introduce contamination and also takes up time, is avoided. Again about 200µl of culture is screened with the clip-on microscopy mount whereas only about 10µl of media can be observed with the traditional wet mount microscopy. A positive culture with lower organism counts will therefore be easier to find with the InPouch system. Also, whilst unused prepared traditional media have to be refrigerated, the unused InPouch can be stored at room temperature, thus appealing to centres with limited refrigeration facilities. Whereas the shelf life of the unused InPouch system is one year, a tube of Diamond's medium has a shorter expiration date (2 months at 4°C).

The InPouch system has been used in studies in Africa [10, 88, 125].

The main differences between the plastic envelope system and InPouch system are that while the InPouch has 2 chambers, the PES has one. InPouch already contains liquid medium whilst that of the PES is dry and has to be reconstituted with water before use.

2.8.4 Staining techniques

To improve the sensitivity of direct smear by wet mount microscopy and also benefit from the prompt observation unlike culture, staining of parasites in fixed and unfixed smears was introduced. Stains such as safranin, methylene blue, malachite green and brilliant cresyl blue do not stain *T.vaginalis* directly. They stain cellular material in the background and permit *T.vaginalis* to stand out against the coloured background. This indirect staining method has not been shown to improve the detection rate of *T.vaginalis* in secretions and thus has not been much used [213].

Fixed smear staining of *T.vaginalis* with Romanowsky stains (Leishman and Giemsa), Gram's stain, acridine orange stain, Papanicolau stain and periodic acid - schiff stain has been done.

2.8.4.1 Acridine orange stain

Acridine orange, a fluorochrome dye, stains the nucleic acid and differentiates DNA from RNA in cells. DNA stains yellow green and RNA brick red under ultra violet light. *T.vaginalis* appears as pear shaped with a yellow green nucleus against light green fluorescence of epithelial cells and polymorphs. Acridine orange stain results compare favourably with culture, detecting 97 out of 120 samples as against 93 of culture [214], and 44 out of 100 samples against 51 of culture in another series [215]. However, the staining procedure is lengthy and the requirement of a fluoresecent microscope puts it out of the reach of many settings. Also, fluorescence of the trichomonads may be difficult to differentiate from cellular matter, thus posing a problem with specificity [216].

2.8.4.2 Romanowsky stains

Leishman and Giemsa stains have been used in fixed smears for over half a century. The trichomonads stain bright blue with darker staining nuclei. More infections have been diagnosed with these than wet film and in a series in which 5 infections were detected by culture, these stains detected the same number, thus equalling the sensitivity of culture [217].

2.8.4.3 Gram's stain

The Gram stain procedure, used routinely in bacteriological diagnosis in many settings including STD clinics has also been used in the diagnosis of *T.vaginalis*. Cree [218] detected *T.vaginalis* in Gram stained smears of 66% of subjects with culture proven trichomoniasis. The false positive rate was 7%. This method detected twice

as many patients with trichomoniasis than wet film examination in another study [219]. However, the sizes and shapes of the parasites vary, sometimes resembling polymorph leucocytes. Not many workers have thus found it useful.

2.8.4.4 Papanicolau stain

Papanicolau (Pap) staining is routinely used in gynaecology settings to detect cytologic abnormalities of the cervix. In Pap stained smears, *T.vaginalis* in secretions appear ovoid with a grey-green cytoplasm and a blue stained nucleus. The efficacy of Pap smears in the diagnosis of *T.vaginalis* infection remains conflicting. While some workers have found the sensitivity comparable to wet preparation [220, 221], others have found it to be more reliable and superior to culture [222]. The specificity of the Pap stain has been questioned by Perl [223]. He could not confirm infection (by culture) in 37% of 666 women whose Pap smears were thought to contain trichomonads. Mason and colleagues could also not confirm infection by other diagnostic methods in 126 women whose Pap smears had been reported as positive for *T.vaginalis* [71]. Pap smear microscopy for the detection of trichomoniasis suffers in general from high rate of false positives (30-48%) [224, 225, 226]. Pap smear microscopy as indeed in all microscopy depends on the experience of the microscopist. The routine staining procedure takes time and the organisms are often difficult to see and distinguish from other cells. Its use solely for the detection of *T.vaginalis* and as the sole method for the diagnosis of trichomoniasis is questionable. In Western urban societies where cervical cytology screening is routine, opportunistic detection of *T.vaginalis* by Pap smear has helped in treating many women infected by the parasite. Bowden has proposed that in urban Australia, introduction of this routine procedure in the 1970s

and 1980s could be responsible for a reduction in prevalence of *T.vaginalis* in those populations [124].

A major limitation of staining methods is that *T.vaginalis* does not always appear in its typical pear-shaped form with flagella. It may be seen as rounded forms resembling polymorphonuclear leucocytes or dysplastic epithelial cells [223, 226]. Sometimes, the typical morphology is lost during fixation and staining, thus making identification difficult.

2.8.5 Serological techniques

2.8.5.1 Serum antibodies

The search for serological diagnosis of *Trichomonas vaginalis* antibodies in the sera of infected patients has gone through many phases. Complement fixing antibodies as used by Trussel [100] and Hoffman [227] detected 48-80% of women with trichomoniasis. It had a poor specificity however, detecting similar antibodies in the blood of 10-40% uninfected patients. McEntegart, described a passive haemagglutination test for *T.vaginalis* detection in 1952 [228]. It detected infection in 42 of 50 infected women and 3 of 50 uninfected women. Using an indirect immunofluorescent test, antibody reactive to *T. vaginalis* was detected in sera of 19 subjects with trichomoniasis but not in 9 uninfected subjects [229].

Detection of serum antibodies to *T.vaginalis* using Enzyme immunoassays (EIA) has also been described. IgG antibodies were detected in 68% of 41 infected and 12% of 128 uninfected women. IgM antibodies, denoting recent infection was found in 22% and 3% of infected and uninfected women respectively [230].

Serological antibody diagnosis has a lot of inherent disadvantages. Serum antibody production depends on the nature of the antigen or its pathogen, whether it is live or

inactivated, its local concentration, and the frequency and length of immune system stimulation. An antibody response may not be observed because the test system is too insensitive to detect low levels of specific antibodies, or because the serum humoral response has not yet been elicited. Trichomonal antibodies may also persist for a long time after treatment or spontaneous cure [231]. The persistence of this serological response and the lengthy procedures involved militate against the use of serum antibody diagnosis in routine trichomonas diagnostic work.

2.8.5.2 Secretion based antibodies

The use of trichomonas specific antibody in secretions has been studied. After various unsuccessful attempts to demonstrate local antibodies in persons harbouring *T.vaginalis*, Ackers *et al* [232] demonstrated the presence of IgA antibody to *T. vaginalis* in vaginal secretions employing ^{125} I-labelled radioimmunoassay. They detected 76% of 29 infected and 42% of 19 apparently uninfected women using their assay. Using antigens prepared from 3 isolates of *T.vaginalis* in an EIA format, Street *et al* [230] detected IgA, IgG or both in cervicovaginal secretions of 73% infected women and 41% uninfected women attending an STD clinic. Su in1982, also detected IgG in 70.8% infected and 23.3% uninfected women respectively [233]. Anti-parasite IgA, IgE, and IgM was also found in 2, 3, and 1 patient(s) respectively, in Su's series.

The persistence of antibody from previous or latent infections could explain the high proportion of antibody positive uninfected women, as also seen for serum antibody [230].

The possibility of cross reacting antibodies to *Pentatrichomonas hominis* cannot be ruled out. Common antigens existing between *P. hominis* and *T. vaginalis* have been

reported [234, 235]. Natural antibodies against *T. vaginalis* and *P. hominis* in sera of normal uninfected people have also been reported by Honigberg [236] and Su [233] respectively.

The poor sensitivity and specificity of these tests and the failure to detect antibody in male secretions [237] does not make these secretory antibodies useful in the diagnosis of trichomoniasis.

2.8.5.3 Antigen based tests

Demonstration of antigenic variation in the surface carbohydrates of *T.vaginalis* [238] and earlier reports identifying type specific antigens of *T.vaginalis* using polyclonal and monoclonal antisera [235, 239], stimulated the development of tests to directly identify *T.vaginalis* antigens in clinical specimens. Using 2 broadly reactive monoclonal antibody panels, Krieger *et al*, identified 88 strains of *T.vaginalis* obtained from diverse geographic areas in the United States and Canada [240]. Lisi and colleagues employed a monoclonal antibody specific for a 65kDa surface peptide of *T. vaginalis* as capture antibody in a sandwich EIA format, and reported a sensitivity and specificity of 89% and 97% respectively compared to culture in vaginal exudates[241]. Other surface peptides to which monoclonal antibodies can be raised and used in such assays are the 200kDa cell detaching factor and cysteine protease, both of which are found on all isolates of *T.vaginalis* [242].

Diagnostic formats using monoclonal antibodies to identify antigens of *T.vaginalis* in vaginal secretions include latex agglutination, enzyme immunoassay (EIA), immunofluorescence, and lateral flow techniques.

Carney *et al* compared latex agglutination with saline wet mount, culture and enzyme immunoassay (with *T.vaginalis* antibody in the solid phase). With culture and/or wet

prep as expanded gold standard, results of the latex agglutination were identical to the EIA at 95% sensitivity compared to74% for wet mount microscopy. Specificities of the EIA and latex test were 99% [22]. Another antigen based point of care test in lateral flow format, approved by the FDA purporting to be more sensitive than culture using vaginal swab eluates has been developed by Xenotope Diagnostics Inc, US. In the documentation submitted for FDA approval, the company reported a100% sensitivity and 98.1% specificity using a composite reference standard (compared the test to wet prep, and resolved discordant samples with culture results). However an evaluation in 2 other centres in the US reported sensitivities of 76.7% and 79.4% and specificities of 99.8% and 97.1%. Culture was the reference standard [243]. The performance of this test in warm and humid environments where the stability of the gold conjugate would be tested is awaited.

Krieger *et al* compared conventional wet mount examination with cultures, monoclonal immunofluorescent antibody staining of direct specimens and cytology in the diagnosis of *Trichomonas vaginalis* [244]. With culture as gold standard, the sensitivities of the wet mount, Pap smear and monoclonal staining were 60%, 56% and 80% respectively, and specificities 99-100%. Comparing the performance characteristics of direct fluorescent antibody (DFA) staining, acridine orange staining and microscopy to culture of vaginal wash specimens in consecutive patients attending a sexually transmitted diseases clinic, Bickley *et al* reported a sensitivity of 83% by DFA, 66% by acridine orange and wet mount. Specificities ranged from 98-100% [105]. Smith has reported a similar sensitivity of 80.6% for the DFA [245].

While the specificities of these tests, with culture as gold standard, have been comparable, the fluorescent staining method surpasses all in its sensitivity.

Unfortunately these tests have not been adapted for routine use. Some of these eg DFA are technically demanding and costly, precluding their use in resource poor settings.

2.8.6 DNA amplification techniques

These are techniques that amplify minute amounts of nucleic acids, to make detection easy. The polymerase chain reaction (PCR), one such technique, is being used for TV detection.

PCR uses the enzyme DNA polymerase, a variant of which is found in the nucleus of all replicating cells. DNA polymerase duplicates DNA during the cells' preparation for divison. In 1983, Mullis realised that exponential growth in the number of copies of a target DNA sequence could be achieved in vitro if several rounds of repeated DNA-polymerase catalysed duplication were made to occur back to back [246]. To undertake PCR, sample is added to a reaction mixture containing an excess of deoxyribinucleotide triphosphates (dNTPs), heat stable DNA polymerase, and 2 primers. These primers are short synthetic oliginucleotides that flank the target region to be amplified. One primer is complimentary to the sense (+) strand, and one to the anti-sense (-) strand at the opposite end of the target sequence.

PCR involves 3 phases, all of which take place in the same reaction vessel, but at different temperatures. The mixture is first heated to temperatures between 90-95 ^{0} C to denature the target DNA. The temperature is then lowered to allow primers to anneal to their respective binging regions. The mixture is heated again to enhance the activity of the thermostable DNA polymerase, which catalyses the extension of each chain from the 3' end of its annealed primer to produce 2 double stranded copies of the target sequence. Both of the 2 newly synthesised primer extension products

contain the appropriate primer-binding regions, and after further heat-induced separation from the target, can themselves function as templates, alongside the original templates, in the next round of duplication. Repetitions of this denaturation, annealing, and extension process, results in exponential accumulation of the target.

Even though the optimal extension temperature for the DNA polymerase is approximately 72°C, the polymerase has activity at room temperature [247].Thus, non-specific products are often generated during PCR set up as well as at the start of thermal cycling, when reactions are briefly incubated at temperatures well below the annealing temperature [248]. Once these non-specific products are formed, they can be efficiently amplified. To prevent this from happening, a hot start procedure is adopted. The hot start procedure delays DNA synthesis by withholding one of the essential components until the thermal cycler reaches the denaturation temperature. This can be done by delaying the addition of the polymerase or using wax barriers to encapsulate essential components such as magnesium or polymerase. Melting of the wax during thermal cycling releases and mixes all of the components together.

PCR is now automated in a thermal cycler which rapidly and reliably changes the temperature of the reaction vessel to provide appropriate conditions for each stage of the amplification process. It is best suited to the detection of DNA of fastidious and non-culturable infectious agents, as it does not rely on the presence of viable organisms in the samples. The first STI related pathogen for which a PCR-based detection system was developed is *Chlamydia trachomatis* [249].

PCR is susceptible to contamination because it is a sensitive amplification technique. Small amounts of contaminating DNA from an exogenous source can be amplified along with the desired template. A common source of contamination occurs when previously amplified products are introduced into new amplification reactions. This

is called carry-over contamination. Purified DNA from other samples and cloned DNA are also sources of contamination. Carry-over contamination can be minimised by using good laboratory procedures during PCR. This includes the use of aerosol barrier tips to prevent aerosols from reaching the pipette barrel, designating separate areas for PCR sample set-up and post amplification analysis, and the changing of gloves before preparing new reactions. The use of premixed reaction components instead of addition of each reagent to individual reactions does also limit contamination. To check for contamination, a negative control without template is always performed.

Several assays using nucleic acid amplification techniques for the diagnosis of *T.vaginalis* infections have been published.

Using primers (TV1/2) targeting a region of the 18S rRNA gene in a polymerase chain reaction (PCR) assay, Mayta *et al* [250] reported a sensitivity of 100% and specificity of 98% with culture as the reference test. This result was obtained by examining 372 clinical vaginal swabs, in which culture detected 24 TV isolates whilst PCR was positive for 31 samples including the 24 detected by culture.

Madico and colleagues [251] designed a primer set (BTUB 9/2) targeting a well conserved region in the beta-tubulin genes of *T.vaginalis*. All strains of *T.vaginalis* tested were successfully detected by PCR, giving a single predicted product of 112 bp in gel electrophoresis. There was no cross reaction from other trichomonads and *N. gonorrhoeae* and *C.trachomatis* which were tested for specificity.

Primers specific for a 102bp region of *T.vaginalis* genome were used by Heine *et al* [252] to detect 91.8% of 61 women with *T.vaginalis infection* (detected by wet prep or culture or PCR) compared to 80.3% by wet mount and culture. Specificity of the

primers for TV diagnosis was 95.2%, also with wet prep and culture as gold standard.

Riley *et al* [253] used a primer pair, TVA5-TVA6, to positively identify 15 cultured clinical isolates of *T.vaginalis* and nine vaginal swab eluates that were wet mount positive for *T.vaginalis*. The clinical isolates were from symptomatic and asymptomatic cases from diverse geographic areas in the US. The TVA5/6 primer pair detects an A6p target sequence which is conserved in *T.vaginalis*.

Shaio, Lin and Liu [254] developed a colorimetric one tube nested PCR assay to detect *T.vaginalis* in clinical specimens. Their assay targeted a family of 650bp specific DNA repeats in the genome of the organism. Out of 378 clinical vaginal specimens tested for *T.vaginalis*, the PCR assay detected 31 as did culture of the specimens. Only 17 of these patient samples were positive by wet mount examination. No specimens negative by this PCR assay were positive by culture or wet mount. There was no cross reaction with human DNA or from other infectious specimens containing *P. hominis* and *Giardia lamblia.*

A set of primers which has been used in many studies from Africa was described by Kengne *et al* (TVK3/TVK7) [255]. This targets a conserved region in the 2000bp repeated DNA fragment of *T.vaginalis* producing a 300bp product.

Defining a true positive as wet mount, culture or 2 PCRs positive, Wendel *et* al [256] reported sensitivities of 52 %, 78%, and 84% for *T. vaginalis* in a study comparing wet prep, culture and PCR respectively on clinical vaginal swabs. Their study indicated that if the women had been offered metronidazole treatment based on PCR results instead of the traditional test results, 84% as against 69% of 97 women would have been treated. They reported lower sensitivities for the PCR primer pairs

(BTUB9/2, TVK3/7 and AP65 A/B) than originally described, ascribing the difference to extended storage of the swabs and repeated thawing.

In a comparison of culture and 5 different primer sets (TVK3/TVK7 [255], TVA5/TVA6 [253], BTUB9/BTUB2 [251], TVE650-1 [254], TV1/TV2 [250]) (expanded gold standard- culture and/or 2 PCRs positive) for the diagnosis of trichomonas vaginitis using self collected vaginal samples from female sex workers in Cote d'Ivoire, Crucitti *et al* [257] obtained much lower sensitivities (59-88%) than reported by studies from the original developers of the assays. They ascribed this difference to different study populations and probably strain variation. The primer sets of Kengne [255] and Madico [251] performed much better than those of Riley [253] and Mayta [251]. The sensitivities of all primer sets improved with the use of enzyme immunoassay amplicon detection compared to gel electrophoresis.

While *Trichomonas vaginalis* PCR assays are not yet commercial as those for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, they nevertheless hold promise for molecular based diagnosis of *Trichomonas vaginalis* infections.

Table 2.2 *Trichomonas vaginalis* PCR primer sets

Primers	Target	Product Size(bp)	Reference
TVK3/K7	2000bp DNA repeats	300	232
TVIP1/2	650bp DNA repeats	290	231
TVA5/A6	A6p	102	234
TV1/2	18S rRNA	312	228
BTUB 9/2	β tubulin gene	112	229

Some of the above discussed laboratory diagnostic tests have the potential for use in resource constrained settings. An evaluation of their diagnostic performance would be helpful in informing choice.

2.9 Treatment of Trichomoniasis

2.9.1 History of treatment of Trichomonas vaginalis

Though *Trichomonas vaginalis* as a pathogen was discovered in 1836 [14] and known to cause vaginitis in 1916 [17], it was not until half a century later that an effective treatment was found for its cure. Before this, the infection was left untreated or treated with a variety of intravaginal preparations including agyrol, kaolin, lactic acid, arsenical compounds, maphasen, mepacrine, aureomycin and trichomycin [258]. Some of these preparations apart from being toxic were cumbersome to use by both patients and physicians. In 1959, a woman died in a London hospital from an overdosage of these treatments [258].

Forgan, writing about the difficulties women went through with these preparations noted, "patients suffered much at the hands of many physicians, and if the last state of the woman was not worse than the first, it was not much better" [258]. A comparative study by Clark *et al* of these treatments showed cure rates of only between 22% - 40% [259]. The treatments also did not eradicate infection in periurethral glands, indicating the necessity for the use of systemic therapy. There was no treatment for men then.

The French Pharmaceutical company, Rhone Poulenc in 1954, screened various antibiotics, antimalarials and amoebicides in search for an anti-trichomonicide. A strain of the mould *Streptomyces*, which produced a substance with anti-trichomonicidal activity, was found in a soil sample from the island of Réunion.

Further manipulation of the chemical structure of this substance called azomycin (2-nitro-imidazole), led to the synthesis of metronidazole (1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole) which was found highly active against *Trichomonas vaginalis* in 1956 [260]. This was followed soon by clinical reports of its use in France and England[261, 262]. Durel and colleagues, who reported this use, did not fully comprehend its value, modestly claiming that its oral use was an adjunct to any local application!

Rhone Poulenc marketed metronidazole in France in 1960 under the proprietary name Flagyl. Concerns about potential toxicity led to a delay in its use in America. These concerns were related to the presence of a nitro group in the drug that was similar to that in chloramphenicol and also to reports of leucopaenia associated with its use [263]. Another reported reason for non use by American physicians was the fear that with its use, the treated women would get better thus preventing the physicians from making money from repeat visits by these women [258]! Thus, it was not until 1963 that it was licensed for use in the US. Today, metronidazole is the drug of choice recommended in many countries for the treatment of both male and female trichomoniasis. It is cheap, easy to produce and a safe and effective drug.

2.9.2 Treatment regimens

Various treatment regimens of metronidazole have been used in the therapy of genital trichomoniasis. The commonly studied and used regimens have been the multiple 200/250 mg, 8 hourly for 7 days dosage and the single 2g dose. Durel and colleagues [262] recommended the 200/250mg, 3 times a day for 7 days treatment. This was shown by Rodin *et al* [264] , McGill *et al* [265] and Watt and Jennison [266] to give cure rates of 85-98% in outpatient female populations. In incarcerated female groups, cure

rates are higher at 90-98% [98, 267, 268] probably due to better compliance and/or fewer opportunities for re-infection. The lower cure rates in outpatient populations can be ascribed to problems with compliance or re-infection from untreated partners. Moffet and colleagues [269] considered that only half those on the multiple dose oral regimen adhere correctly to treatment. Even in clinical trials of this regimen, the percentage of patients failing to attend follow up to ascertain cure and compliance tended to be high – 21%, 22% and 17% respectively [269, 270, 271].

To simplify treatment and improve compliance, the single 2 g regimen was studied and compared to the multi dose regimen. Cure rates between 82-88% [114, 272, 273, 274] for the 2g regimen has been reported in women. Although lower than that for the multi dose regimen in most of the studies, this did not approach statistical significance. Cure rate reports of the 2g regimen in men has varied between investigators. While many report it as good, there is one report of a 43% failure from Zimbabwe [275]. Presently, both the UK [276] and CDC [277] guidelines for the treatment of male and female trichomoniasis recommend the single dose as treatment of choice. This regimen minimises non-compliance, uses 62% less drug, and seems more practical for treatment of sexual partners. It does not however provide protection for prompt re-infection by untreated male sexual partners and is associated with slightly more side effects.

The standard treatment guidelines of the Ministry of Health, Ghana [278] recommends the use of the multi-dose regimen. The clinical efficacy of the single dose regimen has not been evaluated in the country.

2.9.3 Sexual partner management

Due to the high efficacy of the nitro-imidazoles in the treatment of vaginal trichomoniasis, and also high compliance with the metronidazole 2 g single dose

(this can be given as directly observed therapy), failure to treat sexual partners is thought to be the main reason for re-infection in women [279]. Thus cure rates in women have been reported in observational studies to be higher (up to 95%) when the male partners are concurrently treated and only 82-88% when partners are not treated [114, 272]. This is an improvement of 7-13% in women whose partners are treated over those whose partners are not traced and treated.

The CDC guidelines [277] on partner management recommends treatment of all male partners of infected women without recourse to any investigation while the UK guidelines [276] recommends to investigate the partner for other STDs but go ahead and treat for trichomoniasis irrespective of the result of the investigations. Both guidelines thus recommend epidemiologic treatment of all partners of women who have trichomoniasis. However, in Dyker's observational study [272] which is often cited to support epidemiologic treatment, no significant difference was found in reports of sexual partner treatment between patients who failed treatment compared to those who were cured. Likewise, whether women admitted to sexual intercourse or not during treatment was not significantly more common among women who failed therapy than those who were cured. With the knowledge of the natural history of trichomoniasis in men i.e. that spontaneous resolution of infection can occur in up to 80% of infected men, [94, 280] this recommendation of epidemiologic partner treatment for partners of women with trichomoniasis without recourse to results of laboratory investigation may need a re-think. In many developing countries, partner notification for sexually transmitted infections is difficult and may expose index cases, especially women to the risk of violence and marital discord. The knowledge of a male spouse of a partner having a sexually transmitted infection which can be ascertained he does not have (due to spontaneous resolution) can bring untoward social problems to the

woman [281, 282]. Partner notification should only be attempted when it has unequivocally been shown to reduce the risk of re-infection and sequelae.

Though metronidazole is cheap and associated with only minimal side effects, its unwarranted administration to an uninfected partner could have psychological implications.

In another observational study comparing men with *T.vaginalis* infection receiving either metronidazole or tinidazole (another nitroimidazole) at 2 different time points (39 given metronidazole initially and another 34 given tinidazole at another time), Kawamura reported 100% cure rates for these men and that wives of these men cured of trichomoniasis, were less likely to have recurrence of infection after their husbands had been given either drug [283].

Only one randomised control trial using tinidazole in the epidemiologic treatment of male partners of women with trichomoniasis has been reported [284]. This trial showed a significant difference (5% vs. 24%, $p<0.05$) in re-infection in women whose partners received tinidazole as against placebo. However, 9 women in the final cured group had had no sexual intercourse between initial test of cure (when they were found negative for TV infection) and the final evaluation test of cure. So they had not been exposed to any re-infection. Also, there were differences in the observation times (4-6 weeks) for the women from partner treatment to the final evaluation test of cure. This study is also often cited to support epidemiologic treatment of partners of women infected with *T.vaginalis*. While both metronidazole and tinidazole are nitroimidazoles, and there is no reason to suggest metronidazole will not produce similar results, a trial using metronidazole in which subjects have same observation times and whether or not subjects have sexual intercourse between initial and final evaluation is taken into account could be important. This could also be important as

there have been anecdotal reports of success in treating metronidazole resistant *T.vaginalis* isolates successfully with tinidazole [285] implying that tinidazole could have a better cure rate than metronidazole. In countries where metronidazole is the only licensed drug for the treatment of trichomoniasis e.g. the US, this could inform choice.

2.9.4 Trichomonad drug resistance to metronidazole

Antimicrobial resistance to metronidazole was first reported soon after its use in 1962 [286]. The responsible *T.vaginalis* isolate showed decreased in vitro susceptibility to metronidazole, and the patient was eventually cured with double the usual dose. Pereyra and Lansing [268] reported metronidazole resistant trichomonas vaginitis in a prison population, eventually treating these with a repeat oral multi dose regimen supplemented by intravaginal tablets.

It took physicians sometime before associating altered metronidazole susceptibility to intractable clinical disease. The reason had always been ascribed to poor gastrointestinal absorption of the drug and drug inactivation by bacteria [286].

To date, there have been over 100 metronidazole resistant *T.vaginalis* isolated from the US and over 20 from Europe [287, 288, 289, 290, 291] . Some preliminary reports have also come from Nigeria [292], Africa. Many of these infections ultimately resolved with larger doses of metronidazole orally, and intravaginally [293]. It appears that metronidazole resistance in *T. vaginalis* strains is relative and not an "all or none" phenomenon. Resistance varies from low to high level, and most cases can be cured with increased dosages of the drug. Concomitant intravaginal treatment with pessaries or suppositories could be helpful in the resolution of infections.

In many developing countries including Ghana, metronidazole can be bought over the counter, and suboptimal doses are often consumed by patients. It would not be unusual for antimicrobial resistance to develop to it. There is a high rate of abuse of antimicrobials used in sexually transmitted infections clinical practice in Ghana [294], with resulting high drug resistance profile of the gonococcus [295]. The continued clinical efficacy of metronidazole against *T. vaginalis* warrants studying.

2.10 Summary

Recent studies indicate that the control of trichomoniasis could be important at the community level in the control of HIV/AIDS in countries where both conditions are endemic. It could also be important in reducing adverse pregnancy outcomes in such endemic countries. Presently, control measures depend on the sexual transmission of *T.vaginalis*. There have been speculations in recent times that gut related human trichomonads may play a role in the aetiology. Information is needed on the role if any of the gut related *P.hominis* in this aetiology and also that of the oral *T.tenax*. Such information could be important as these human trichomonads to a large extent may not be transmitted sexually. Whether or not some TV strains have their origin in the rectum could be important in prevention programmes.

Statistical modelling indicates that the control of *T.vaginalis* infection would be better served by screening and treating populations of the infection instead of the present syndromic management approaches used in many resource-poor settings. This however depends on the availability of a diagnostic test with good test characteristics that could be used under conditions in these settings. The evaluation of some of these diagnostics would inform choice.

In the absence of a good and cheap diagnostic for *T.vaginalis* infection, elucidating epidemiological and clinical factors that could predict infection would be helpful in selecting patients for screening and treatment of infection.

These issues are examined in these case-control and diagnostic comparison studies.

3. 0 METHODS

3.1 Study sites

The study was carried out in the Kumasi metropolis of the Ashanti Region, Ghana (Fig. 3.1). Kumasi is the capital of the Ashanti Region in Ghana and it is the second largest city in the country. It is located in the tropical rainforest of the country. With an area of 150 sq km, it is 275 kilometres up north from the capital. Kumasi has a rich ancient traditional history and culture.

Kumasi is the largest of the 18 political districts in the Ashanti region, and is bounded by Kwabre district to the north, Bosumtwe- Atwima- Kwanmoma district to the south and Ejisu-Juaben and Atwima districts to the west and east respectively. Politically, the city is divided into four sub-metropolitan areas namely, Manhyia, Asokwa, Bantama and Subin. For health service delivery, the Manhyia sub-metroplitan area is divided into Manhyia North and Manhyia South.

The 2002 population census in Ghana estimated the population of Kumasi at 1.2 million inhabitants, with an annual growth rate of 2.6% [296]. The daytime population could be much higher as Kumasi is the hub of trading activities within the region, between the northern half of the country and the south, and lies on the main highway linking the capital, Accra to other West African countries such as Mali, Burkina Faso, and Cote d'Ivoire.

As a cosmopolitan city, Kumasi attracts people of major ethnic groupings in West Africa. The indigenous Asante people and other Akans dominate life in general, especially in commerce and industry. It is believed the Asantes dominate business and trading activities in the country. People originating from the north of Ghana (northerners) have over many years lived and maintained identifiable communities in Kumasi. Their population has increased in recent times with rural-urban migration.

Clearly defined areas of the city are also inhabited by migrant communities from the Volta region of Ghana and other neighbouring West African countries. Although these non indigenous Akan communities maintain their language and cultural identity, Asante Twi, the language of the Asantes, is universally spoken.

Health Services in Kumasi are organised around 5 sub-metro Health teams, each with a recently upgraded hospital. These are the Manhyia South sub-metro with the Manhyia hospital, Manhyia North Sub-metro with the Tafo hospital, Subin sub-metro with the Maternal and Child Health Hospital and the Bantama sub-metro with the Suntreso Hospital, and also the Kumasi South Hospital. The sub metropolitan health teams, led by a Metropolitan Director of Health Services, have the responsibility of planning, implementing, monitoring and evaluating health services in the Kumasi Metropolis.

The Komfo Anokye Teaching Hospital (KATH), one of 3 public autonomous teaching hospitals in the country is sited in Kumasi. It is a national tertiary referral hospital which serves the northern half of the country.

Apart from these public hospitals, there are other health facilities in the quasi Government and private sector in the metropolis including 50 hospitals, 79 clinics, 18 maternity homes, and 20 homeopathy clinics.

The study was located in 4 sites; the Suntreso, Manhyia and Tafo Hospitals where study subjects were recruited, and KATH where some of the laboratory work was carried out.

The Suntreso and Manhyia hospitals are the largest of the 4 Government hospitals in the city (KATH excluded). Services in both facilities include general outpatient clinical care, Maternal and Child health services including daily antenatal and post

natal clinics, infant growth monitoring and nutritional support clinics, and child immunisation clinics. General and Obstetric surgery is also carried out.

The antenatal clinics of the 3 study hospitals see an average of 100 clients each a day, and these were the facilities used by the study. For study purposes, a mobile microscopy service for vaginal wet preparation microscopy (wet preps) between these centres was set up.

Cultures for *Trichomonas vaginalis*, Gram staining and the reading of gram stains of vaginal smears for the study, and storage of study swabs and *T. vaginalis* isolates was done at KATH. Quality assurance of all the laboratory tests done in-country was carried out at KATH.

Further laboratory work was undertaken at the London School of Hygiene and Tropical Medicine and the Institute of Tropical Medicine, Antwerp.

Figure 3.1 Map of Ghana

3.2 Study Population

These were pregnant women attending the antenatal clinics of the Suntreso, Manhyia and Tafo Hospitals. This population was chosen for convenience and ease to reach. Also, the clinics and their staff have been involved in previous reproductive health research, thus making it easier to launch study protocols there. Results obtained from this population may not be generalisable to other female populations.

3.2.1 Inclusion criteria

Below were inclusion critereia for the study:

i. Study subjects must be pregnant and attending the antenatal clinic of the relevant health facility

ii. They should be able to attend the clinic for at least a week after first being seen

iii. They should be able to give informed consent independently.

iv. They should live within 10 miles radius of the Kumasi metropolis

3.2.2 Exclusion criteria

Exclusion criteria for the study were:

1. known hypersensitivity to metronidazole
2. has been referred to the clinic due to pregnancy and/or birth related problem
3. needs cardiopulmonary resuscitation

3.3 Study design

The study was conducted in 2 stages. Cases of *T.vaginalis* infection were identified by screening of the women attending the antenatal clinics, and then an unmatched case-control study carried out. This was to determine the association between infection and various socio-demographic, maternal, genital hygiene, and sexual behavioural factors. It also looked at the association between infection and various clinical parameters for the diagnosis of infection.

Using swabs obtained for the diagnosis of TV infection, a comparison of various diagnostic methods for the detection of TV was done.

3.4 Sample size calculations

In calculating sample size for this study, it was assumed that the minimum odds ratio of *T. vaginalis* infection to be detected would be 1.7 [10]. Thus, a sample size of 210

cases and 420 controls would have an 80% power to detect a significant effect ($p<0.05$) for a risk factor to which 25% of controls are exposed.

The prevalence of *Trichomonas vaginalis* in the antenatal clinics after pilot study averaged 10%. Thus 2,100 women would have to be screened. Allowing for a 10% loss to follow up, approximately 2400 women would be screened.

3.5 Training

Prior to the commencement of the field work, field workers were recruited and trained in study procedures.

Three main groups of fieldworkers were involved; nurse interviewers, midwife examiners and laboratory technicians.

Training for the midwife examiners involved genital examination, the insertion of specula, visualisation of the vagina and cervix and their reporting and the adequate insertion of swabs and taking of samples from the posterior fornix. The 2 day training was supervised by the study gynaecologist/obstetrician and included discussions and practical hands on sessions with patients. As the examiners were midwifes by training and had been doing this routinely, there was no difficulty with this training.

Training in study laboratory procedures was supervised by the author of this work. The InPouch culture (BioMed Diagnostics, San Jose, Ca) had not been used in the study laboratory previously, so opportunity was taken to train all lab staff in the use and examination of the InPouch. A training video supplied by the manufacturer augmented the training. Staff were trained in wet mount microscopy and the latex agglutination test according to manufacturer's instructions.

The author and Principal investigator of the study had undergone training in London on the reading of Gram films to score bacterial vaginosis according to Nugent's criteria. This training was passed on to one of the technicians.

Three laboratory technicians were trained, who independently and blinded to each other, read the latex agglutination, wet mount and InPouch cultures respectively.

The Principal investigator supervised training of the study interviewers using the study questionnaires. Culturally inappropriate questions were deleted, and agreement reached on uniformity in asking sensitive questions related to sexual practices.

Results of the piloting and pre-testing were used to adapt the questionnaires and organisation of data collection.

To check the quality and consistency of reporting responses from study subjects during the study, the consent of 60 randomly selected participants (about a tenth of participants) was sought to enable all the 3 nurse interviewers sit-in and record responses during their sessions. Responses were then checked.

3.6 Recruitment Methods

Recruitment of study participants began in mid October 2002 after a two week piloting and pre-testing study and ended in August 2003.

At each antenatal clinic, the antenatal cards of the women were examined to check if they had not been recruited previously (a label marked "Trichomoniasis Study" was stuck on the card of all previously recruited women).

Study interviewers (nurse/midwives) read out the study information sheet in the local Twi language to potential study participants. Verbal witnessed consent was then obtained from those who met inclusion/exclusion criteria and agreed to participate in the study. Written informed consent was not taken because of the poor literacy rate

among the women. Even though immediate consent was sought from the women, they were nonetheless assured that non-paticipation would not affect the care they received at the clinics. Study interviewers then instructed women consenting to participate in the study on how to collect self administered vaginal swabs (SAVS). In brief, they were instructed to gently insert the swab into the vagina and swab the vaginal walls. Participants were given dacron swabs (Quelab, Canada) for this purpose. After collection, the SAVS were sent to the on-site study laboratory where the swabs were screened for *T. vaginalis* with the Kalon TV latex test (Kalon Biologicals, Surrey, UK).

A sticker with the label, "Trichomoniasis study" was placed in the antenatal card of these consenting women. This was to prevent re-screening during subsequent visits.

After screening for *Trichomonas vaginalis*, the laboratory personnel passed on all women positive for *Trichomonas vaginalis* infection (by the TV latex test) and the next 2 consecutive women who were negative for infection in a random order to the interviewers. The interviewers were blinded to the results of the *Trichomonas vaginalis* screening test.

Confidential interviews were conducted with a structured questionnaire (Appendix 1) which sought information on a variety of sociodemographic and maternal factors, sexual practices, and ano-genital hygiene practices of study patients, after which the interviewees were passed on to other study staff for genital examination.

3.7 Clinical Procedures

3.7.1 Genital examination and sampling

This was done by trained, experienced midwifes who were also blinded to the results of the TV screening test.

The nature of the examination and the swabs to be taken was explained to study participants. A total of 6 swab samples per subject was taken – 1 oral, 1 rectal and 4 vaginal.

The women were asked to undress and lie on the examination couch. Examination was done in good light.

A dacron swab was used to take material from the periodontum and gingival areas of the oral cavity. The swab was placed in a white labelled tube.

The external genitalia were examined for any discharge, genital ulcer and/or vesicles, and other pathologies. A vaginal speculum was carefully inserted, and discharges and/or ulcers in the vagina and cervix looked for. The cervix was also examined for any pathology, especially punctate haemorrhages (strawberry cervix).

Four (4) swabs from the posterior fornix were taken per subject and put in blue labelled tubes.

On withdrawing the speculum, the pH of any discharge was checked using pH indicator strips (Color-pHast, MC/B Manufacturing Chemists Inc, Cincinnati) and the amine test performed with in-house prepared 5% potassium hydroxide (KOH).

Finally, with care and having told the subject, a rectal swab was obtained by inserting a dacron swab about 2 cm into the rectum and rolling it.

All specimens were sent immediately to the on-site laboratory bench.

Examination findings were recorded on the "Female Clinical Examination Form" (Appendix 2).

3.7.2 Definitions of characteristics of discharge

An abnormal vaginal discharge was defined as one with an abnormal odour, any colour other than clear, any amount other than normal, or yeast-like. The amount of

vaginal discharge observed was categorised as normal (no obvious discharge seen), discharge seen within the vagina only and discharge seen at the introitus.

3.8 Metronidazole therapy

At the end of recruitment for each day, standard metronidazole therapy (2g *stat*) was given to all study subjects in whom *Trichomonas vaginalis* had been detected by wet prep microscopy. This was also given to women (visiting the clinic a month after the initial examination) who at the initial visit were wet prep negative, but whose InPouch culture results were positive during the week. The women were counselled to stay off alcohol for 48 hours after ingestion of the metronidazole and also abstain from sexual intercourse for a week until after the test of cure. A prescription for metronidazole was also written to be given to their sexual partners.

All these women were asked to return to the clinic a week after treatment for a final test of cure.

3.9 Final test of cure questionnaire

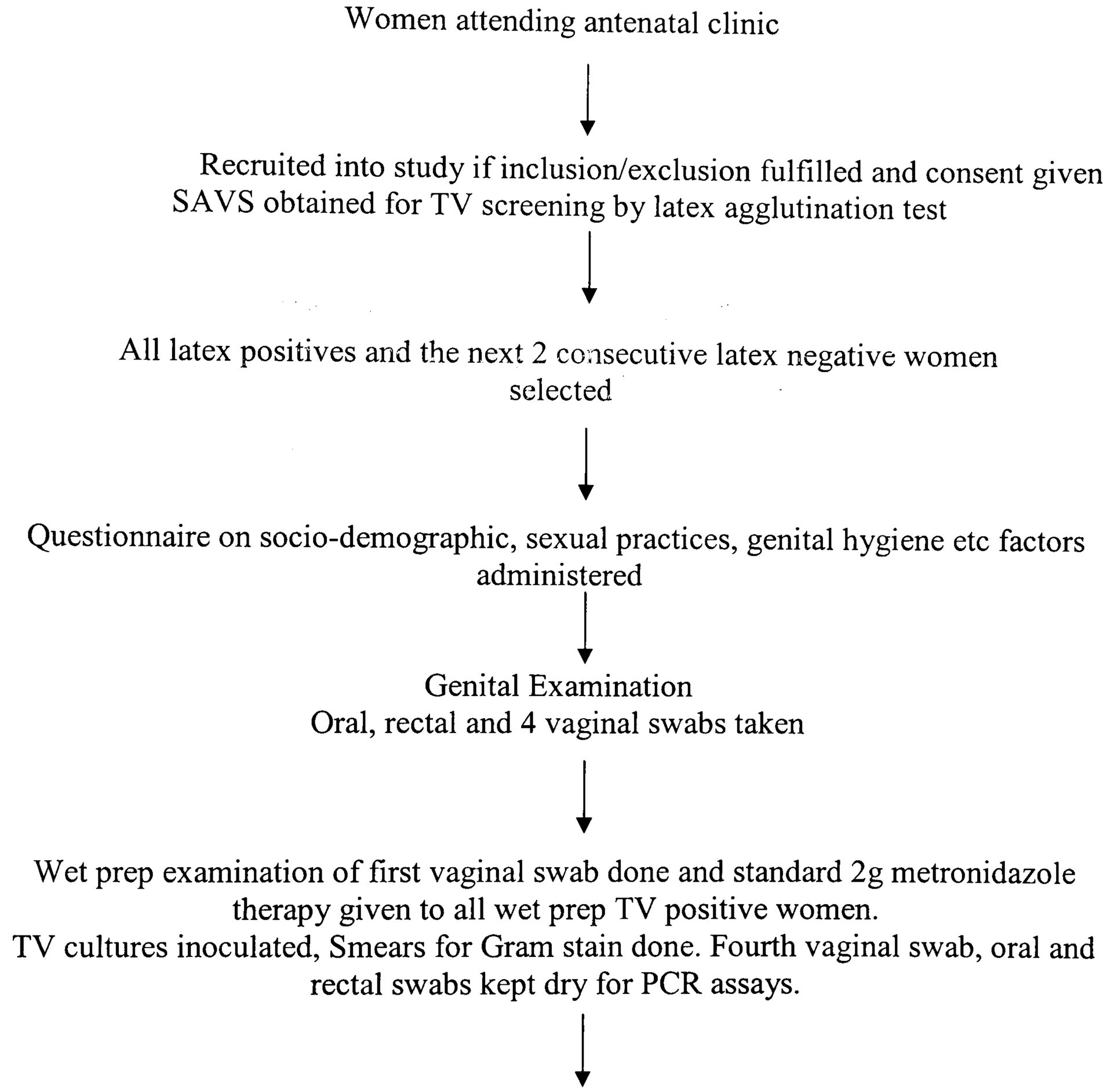
At the one week follow up visit, the women answered a questionnaire on the frequency, if any, of sexual intercourse during the intervening period, whether or not their partners had taken the metronidazole and any adverse reactions to metronidazole intake (Appendix 4).

A SAVS was then obtained for the test of cure. This was done by InPouch culture as described in 3.11.3.

3.10 Storage of swabs

The oral, rectal and fourth of the four vaginal swabs were kept dry in sample tubes at the recruiting clinics and sent to the KATH within 30 minutes to an hour of collection where they were stored at -20^{0}C. These were subsequently shipped on dry ice to London and Antwerp, and used in nucleic acid amplification tests.

Figure 3.2 Flow chart of study procedures



3.11 Laboratory Methods

3.11.1 Latex agglutination testing

SAVS from recruited patients was screened for *Trichomonas vaginalis* by a latex agglutination kit (LAT) according to manufacturers' (Kalon Biological Ltd, Surrey, UK) instructions.

The TV latex kit consists of the following in dropper bottles; 2.5ml test latex, 1.5ml positive control, and 1.5ml negative control, all in 0.1% sodium azide preservative. Also in the kit are sterile swab sticks, sample tubes containing 500μl phosphate buffer in sodium azide, wooden mixing sticks, and a reusable glass slide.

The test latex is sensitised with rabbit anti-*T.vaginalis* IgG. If the test latex is mixed on a slide with eluate from a vaginal swab, any *Trichomonas vaginalis* antigen present causes cross linking (agglutination) of the sensitised latex. Agglutination, which can be observed against a dark background is indicative of the presence of *T.vaginalis*.

All the TV latex reagents were brought to room temperature from 4^0C storage. The TV latex reagent was shaken to give a good mix to the particles.

The SAVS were eluted into the manufacturer-supplied sample tube containing phosphate buffered saline (PBS). 50μl of swab eluate were added to a reaction zone on the glass slide. One drop of latex was added to the eluate. This was stirred and mixed to a completely homogenous mixture that covered the entire surface of the reaction zone. The glass slide was tilted with a rotating action continuously for 2 minutes. The degree of agglutination obtained, was read after 2 minutes and scored as follows;

Latex has agglutinated and much has collected around edge of reaction zone +++

Agglutinated particles can clearly be seen against a granular latex background ++

Agglutination can just be discerned when compared to negative control +

No agglutination compared to negative control - negative

Positive and negative controls were run each day before study subject samples were run.

All tests scored with any degree of + were recorded positive.

The left-over swab eluates were stored at – 20°C and later shipped to London.

3.11.2 Wet prep examination

The first of the 4 vaginal swabs obtained during the genital examination was immediately agitated into 0.9% saline solution. A drop of this was placed on the centre of a clean microscope slide and covered with a cover slip. The slide was initially carefully scanned at x100 with a light microscope for motile trichomonads, pus cells, yeast cells and epithelial cells, and then at x400, to confirm motility, flagella movement, and morphological features of *Trichomonas vaginalis*. This was done on site in the respective hospitals.

3.11.3 InPouch inoculation and culture

Culture for *Trichomonas vaginalis* for the study was undertaken using the InPouch™ system (BioMed Diagnostics, San Jose, Ca, USA).

The second of the 4 vaginal swabs was inoculated into the InPouch system within 5 minutes of specimen collection. It was observed under the light microscope immediately for any motile trichomonads and then sent to the KATH Clinical Microbiology laboratory within 30 minutes of specimen collection, where it was incubated at 37^0C, and observed daily for up to 5 days for any motile trichomonads.

Microscopy was carried out at x100 and then x400. Pouches not registering the growth of trichomonads after the fifth day were declared negative.

3.11.4 Reading of above laboratory test results

The latex agglutination test, wet mount examination and InPouch culture results were read independently, each by different technician who were all blinded to each others' results.

3.11.5 TV Enzyme immunoassay test

This was carried out on stored left over PBS eluates used originally for the latex agglutination testing and transported to London. A commercial kit, TV screen (Kalon Biological, UK), was used. The kit has polystyrene microtitre plate wells supplied pre-coated with affinity purified polyclonal antibodies to *Trichomonas vaginalis*. Testing was carried out according to manufacturer's instructions.

Vaginal swab eluates stored at -20°C were brought out to thaw at room temperature. 100μl eluates and controls were dispensed into designated wells and incubated at 37°C for 30 minutes. After a wash step with distilled water (4 times washing and tapping plate dry), the surface of each well was probed for antigen by incubation at 37°C with 100μl enzyme conjugated anti-TV tracer. Following a second wash step (4 times washing and tapping plate dry), 100μl enzyme substrate/TMB chromogen was added to the wells. The plates were incubated at room temperature for 30 minutes and the enzyme incubation then stopped with 1N 100μl sulphuric acid. Addition of the stop solution changed the colour of the reaction whose optical density (OD) was then measured in a photometer at 450nm. The optical density is proportional to the amount of TV antigen present in the original eluate.

Assay validation was done for each run according to the manufacturer's criteria i.e cut-off positive control OD is greater than 0.4, the negative control O.D is less than 0.3 and the ratio of the cut-off positive control to the negative control is greater than 1.5.

All samples with an OD greater than cut-off X 1.1 were considered positive and those with OD less than cut-off X 0.9 considered negative. Results with OD between 0.9Xcut-off and 1.1Xcut-off were considered equivocal and repeated.

3.11.6 Lateral flow test (Kalon TV dipstick)

This kit has not been manufactured for commercial use. Kalon Biological Ltd sent in a quantity of the strips to be evaluated with the left over archived swab eluates.

The lateral flow test is a dipstick made from nitrocellulose membrane with mesh sizes of 10 microns. The dipstick has 4 distinct areas; a sample pad area containing dried down phosphate buffered saline on which the vaginal swab eluate is put, a red gold conjugate pad, a diffusion area at the end of which 2 invisible lines are found; an anti-goat antibody control line and an anti-TV control line, and a 'data' area, where sample numbers may be written.

150μl of swab eluate was applied to the sample pad area and allowed to diffuse through the length of the dipstick, over the gold conjugate and the 2 invisible lines for 20 minutes. The anti-goat internal control line shows up red with a valid run. A positive test shows up as 2 red lines, whilst a negative test shows up with the internal control line. The absence of the internal control line indicates an invalid test.

3.11.7 Polymerase Chain Reaction (PCR) for TV

3.11.7.1 Primers

For the detection of *Trichomonas vaginalis*, 2 different oligonucleotide primer pairs, based on their use in clinical and epidemiological studies were employed; one pair designed by Kengne [255], and the other, an internal primer reported by Shaio [254]. These primers amplify independent targets in the *T.vaginalis* genome, producing amplicons of different sizes.

The Kengne pair of primers, TVK3/TVK7 amplify a target within the 2000bp repeat region in the TV genome, producing an amplicon of size 300bp. The Shaio internal pair of primers, IP1/IP2 produce a product with size 290bp after amplifying a target in the family of 650bp specific DNA repeats in the *T vaginalis* genome. Reports from the literature suggest the Kengne pair of primers to have better sensitivity than the Shaio primer pair [254]. Also Studies from Africa have utilised the Kengne primers and reported their favourable sensitivity. All samples were first screened with Kengne primer pair. All those positive, were then further tested with Shaio pair of primers.

Primers used in *P.hominis* amplification are as reported by Crucitti *et al* [11]. These (Th3 and Th5 primers) amplify a unique region of the 16s-like ribosomal RNA gene of *P.hominis* producing a 339 bp fragment.

For *Trichomonas tenax*, a primer pair, PT3/PT7 designed by Kikuta *et al* [297] was used. The primer pair amplify a 776 bp fragment from the 18S rRNA gene of *T. tenax*.

Amplification of the human $ß_2$ gene was also done on all the vaginal and oral swabs to check for the quality of material on the the swabs. Primers used were the PC04 and GH20 [298]. These produce a 268bp amplicon.

All the primers were synthesised by Sigma-Genosys Ltd, UK

3.11.7.2 DNA extraction

DNA extraction from the swabs was done using commercial kits from Qiagen, UK. These were QIAmp DNA mini kit (extraction from vaginal and oral swabs) and QIAmp DNA stool mini kit (extraction from rectal swabs). Extractions from vaginal, rectal and oral swab specimens utilised a similar protocol.

Extraction was done in a Class 2 safety cabinet. Standard precautions against cross contamination were employed. Bench tops, hood and discard pots were cleaned with a 10% hypochlorite solution (Hays Chemical distribution Ltd, Leeds, UK), and 70% alcohol (Sigma UK). Sample racks, and pippettors were similarly cleaned. Aerosol barrier tips were used throughout. Gloves were liberally changed as and when necessary.

The QIAmp kits contain 3 buffers – AL, AW1, and AW2 - used at different stages of the extraction process. Added to the lysis buffer (AL) of the DNA stool mini kit is InhibitEX®, a commercial polysaccharide mixture which removes PCR inhibitors of faecal origin such as bile salts, break down products of haemoglobin and complex polysaccharides often found in food [299, 300].

The fourth vaginal swab (or the rectal or oral swab) was thawed from -20°C storage and eluted in 500μl PBS by vortexing for 10 seconds and squeezing the swab against the inside of the polypropylene sample tube. 250μl of swab eluate was aliquoted into 2ml sample tubes. To this was added 600 μl AL buffer and vortexed for 15 seconds. 20μl Qiagen proteinase K was then added and the mixture incubated in a heating block at 56^0 C for 10 minutes. After cooling, it was centrifuged briefly and 600ul of 96-100% ethanol was added. 700μl of this lysate was aliquoted onto QIAmp spin columns placed in 2ml tubes. The spin columns were centrifuged at 6000g for a minute after which filtrate in the 2ml tubes was discarded. The column was put in a

new 2ml tube. A further 700μl of lysate was dispensed onto the column which was similarly centrifuged and the filtrate discarded. The column was placed in a new 2ml collection tube. 500μl of AW1 buffer was added to column and spun at 6000g for a minute. Filtrate was discarded and column was placed in another collection tube. AW2 buffer (500μl) was added to the column, spun for 2 minutes at 20,000g and filtrate discarded. The QIamp columns were finally eluted with 200μl TE buffer. The resulting eluate, containing any extracted DNA was stored at -20°C until used.

3.11.7.3 DNA Amplification

Amplification of DNA was done in a programmed thermocycler (GeneAmp PCR system 9700, Applied Biosystems, Norwalk, US). All the PCR reagents (buffer, $Mgcl_2$, and polymerase), were obtained from Applied Biosystems, UK, and the bases (dNTPs) from Amersham Corp., UK.

3.11.7.3.1 *T.vaginalis*

Preparation of the PCR master mix was carried out in a dedicated class 2 cabinet (separate from the one used in the extraction process). Similar precautions against cross contamination as above were taken. The master mix for the Kengne PCR consisted of 5μl 10X gold buffer, 7μl 2mM of each dNTP, 1.5μl each of 5uM primers, 4μl 25mM $Mgcl_2$, and 0.4μl 5U/ml amplitaq gold DNA polymerase and 20.6μl distilled water. 40μl of this mix and 10μl of sample DNA extract were put in each of the PCR reaction vessels. A positive control of DNA from a known *Trichomonas vaginalis* reference strain (ATCC 3001) and a negative control of distilled water were substituted for samples in the last and penultimate reaction vessels. Cycling conditions were a pre-PCR heating at 95°C for 5 minutes (hot start),

and 35 cycles of denaturation at 94°C for 1 minute, primer annealing at 60°C for 30 seconds, and product extension at 72°C for 2 minutes.

The Shaio PCR mix consisted of 4μl 10X gold buffer, 4μl 2mM of each dNTP, 4μl each 5uM primers (IP1 and IP2), 3.2μl 25mM Mgcl2, and 0.4μl 5U/ml amplitaq gold polymerase. 40μl of this mix and 10μl of sample or control DNA were used. Cycling conditions were 95°C for 45 seconds denaturation, 45°C for 1 minute annealing, 72°C for 1 minute extension. 35 cycles were run. This also involved a pre-denaturation hot start procedure at 95°C for 5 minutes.

3.11.7.3.2 *P.hominis*

The PCR master mix for *P. hominis* amplification consisted of 5μl 10X gold buffer, 7μl 2mM of each dNTP, 1.5μl each of 5uM primers, 4μl 25mM $Mgcl_2$, and 0.4μl 5U/ml amplitaq gold DNA polymerase and 20.6μl distilled water. 40μl of this mix and 10μl of sample DNA extract were put in each of the PCR reaction vessels. Cycling conditions after a 95°C hot star procedure for 5 minutes, was 1 minute denaturation at 95°C, annealing at 64°C for 1 minute and product extension at 72°C for 1 minute. 35 cycles were undertaken. 40ul of master mix and 10ul sample DNA extract was used in the reaction vessels.

3.11.7.3.3 *T.tenax*

The *T.tenax* programme consisted of 37 cycles of denaturation at 95°C for a minute, annealing at 58°C also for a minute, and a product extension time of 1 minute at 72°C. Cycling was preceded by a 7 minute hot start procedure at 95°C. The reaction mix consisted of 10μl buffer (10X strength), 10μl Mgcl2 (25mM),10μl of each

dNTP (2mM), 3μl of each primer (5uM of PT3 and Pt7), and 1μl Amplitaq gold polymerase. 90μl of master mix and 10ul of DNA was cycled.

3.11.7.3.4 ß-2 microglobulin

Each swab eluate was checked for the presence of human DNA ($ß_2$ microglobulin gene) by PCR for probable inhibitors and also the quality of sampling. All samples which appeared to have inhibitors were diluted ten fold and $ß_2$ microglobulin run again.

The ß-2 PCR mix consisted of 4μl 10X gold buffer, 4μl 2mM of each dNTP, 2μl each of 5uM primers (PC04 and GH20), 6.4μl 25mM Mgcl2, and 0.4μl 5U/ml amplitaq gold polymerase and 1.2μl distilled water. 20μl of this mix and 20μl of sample or control DNA were used. The control DNA was a DNA extract from human lymphocytes (courtesy of Ms.Tania Crucitti, Institute of Tropical Medicine, Antwerp).

The $ß_2$ cycling conditions were; pre-denaturation hot start at 95°C for 2 minutes, then 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, and 30 seconds extension at 72°C.

After the final cycles of all the PCRs a final extension period of 7 minutes at 72°C was undertaken.

3.11.7.4 Amplicon detection

Products from *T.vaginalis* and ß-2 PCR were detected by colorimetry, using Enzyme immunoassay techniques (EIA) [257], whilst those of *P.hominis* and *T.tenax* were detected by agar gel electrophoresis [11,297].

3.11.7.4.1 Capture probes

For detection of amplicons from the Kengne TVK3/7 *T.vaginalis* PCR, a 25 bp DNA probe, SA3 [257] was used while primers TVER-P2 designed by Ryu [301] was used to capture amplicons from the TV IP1/2 Shaio amplification. The 268bp PCR product from the ß-2 microglobulin amplification was detected using a PC03 probe [302].

All the capture probes were synthesised by Sigma Genosys, UK.

3.11.7.4.2 Coating of plates for EIA

Coating buffer for the microtitre plates consisted of freshly prepared ammonium acetate and either 500ng/100μl SA3 probe, 300ng/100μl TVER-P2 probe or 300ng/100μl PC03. 100μl of a 1M ammonium acetate/probe solution was put in wells of a microtitre plate (Nunc, Denmark), covered with a microtitre plate seal and incubated at 37°C overnight. After overnight incubation, the wells were washed twice with 1:10 Amplicor wash buffer (Roche Diagnostic Systems, NJ), tapped dry, covered with laboratory tissue and left to dry, at room temperature for 2 hours. Dried plates were used immediately or stored at 4°C and used within a week.

3.11.7.4.3 EIA run

Similar protocols were used for amplicons from both *T.vaginalis* and the $ß_2$ microglobulin reactions.

Amplicons were heat denatured at 95°C for 5 minutes. 15μl of this was added to a 100μl hybridisation buffer in microtitre wells (above). The plates were covered with sealant and incubated at 37°C for an hour, after which the wells were washed five times with Amplicor wash buffer.100μl Horse Radish Peroxidase (HRP)-streptavidin was then dispensed into each well. The plate was again incubated at 37°C for 15

minutes, after which it was washed five times. 100μl TMB substrate was added to wells in the dry plate and the plate incubated in the dark at room temperature for 10 minutes, after which the reaction was stopped with 100μl 1N H_2SO4. Absorbance was read in a spectrophotometer (MRX Dynatec, UK) at 450nm.

Each plate had 2 positive control wells and 2 negative controls. The cut off was calculated as the mean of the negative controls + 3 standard deviations.

3.11.7.4.4 Agarose gel electrophoresis for *P. hominis* and *T. tenax*

PCR products from the *P.hominis* and *T.tenax* amplifications were analysed by electrophoresis in a 1.5% agarose gel (Life Technologies, UK) in Tris acetate EDTA buffer (pH 8) (Appendix 6). The gel was stained with ethidium bromide (CLP, London). 10μl of product was mixed in 2μl of commercial loading buffer (Bioline Ltd, London) and loaded onto the agarose gel. Electrophoresis was done in a 12X14 horizontal gel tank (Apollo, CLP, UK). After electrophoresis, the gel was visualised under ultraviolet light and photographed (BioImaging Systems, UK). Sizes of the amplified products were assessed by comparing to commercial molecular weight markers (1kb, Bioline Ltd, London). Positive and negative control samples were run alongside sample amplicons.

All PCR analysis was carried out according to quality assurance guidelines for molecular diagnosis [303].

3.11.7.5 Interpretation of results

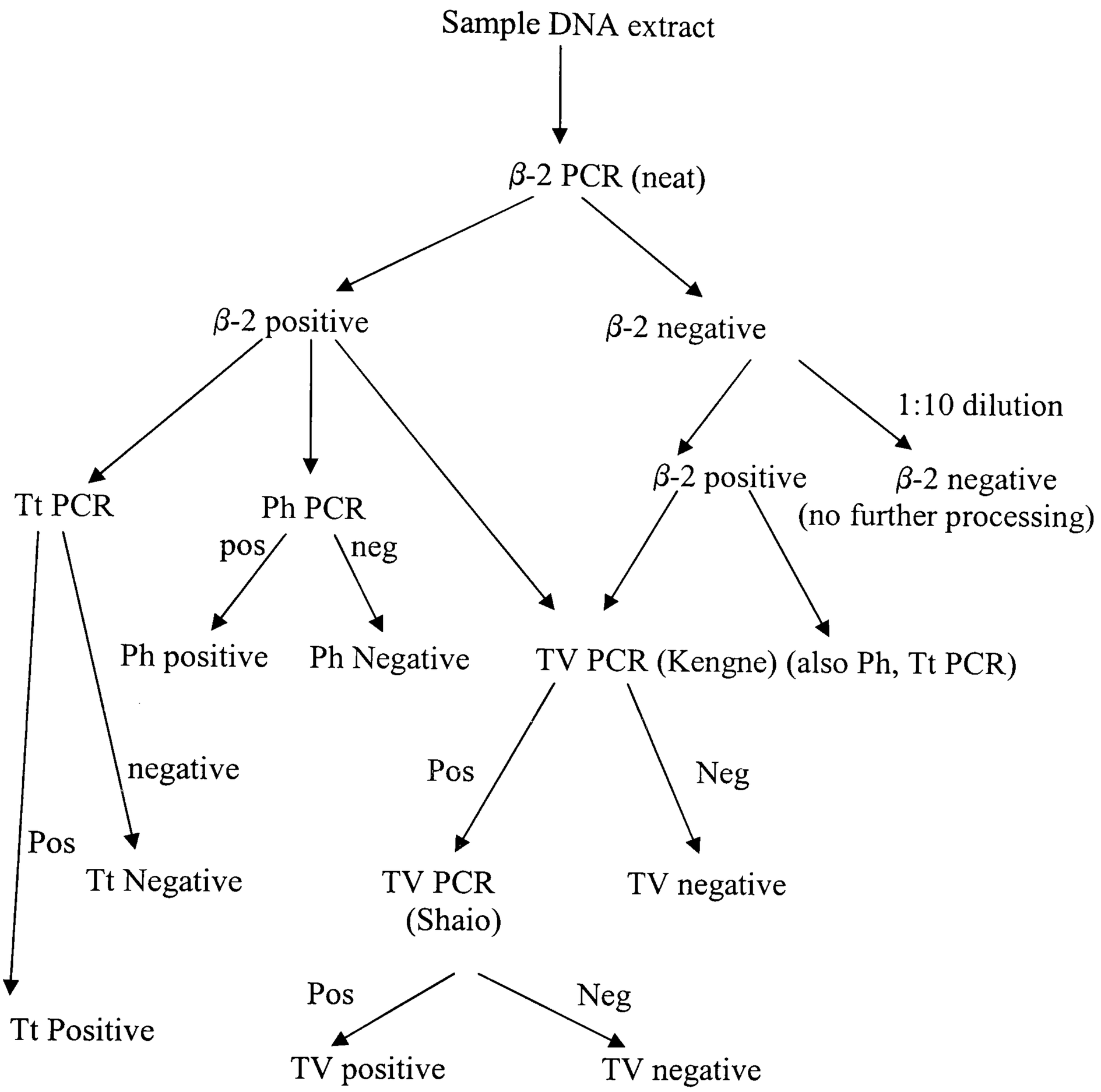
Any samples with values greater than the cut-offs in the EIA analysis were deemed positive, and those with values below the cut-off values, negative.

Samples negative for $ß_2$ microglobulin undiluted and diluted were considered to have PCR inhibitors or have inadequate sample. If after the 1:10 dilution step $ß_2$ testing was still negative no further analysis was carried on those samples. Otherwise, further PCRs were carried out.

All samples positive with Kengne primer pairs were tested again with Shaio primer pairs. A sample was adjudicated positive only if testing with both Kengne and Shaio primer pairs were positive.

A positive gel product was one where electrophoresed product was of the expected size as judged by the molecular weight marker and the positive control.

Figure 3.3 Flow diagram of PCR testing of vaginal swab eluates



β-2: β-2 microglobulin
TV: *Trichomonas vaginalis*
Ph: *Pentatrichomonas hominis*
Tt: *Trichomonas tenax*

3.11.8 Gram Stain preparation and examination

A smear was prepared on a clean glass slide with the third of the 4 vaginal swabs at the on-site laboratory. The smear was allowed to dry in air. This was transported to the Microbiology laboratory of the KATH, where the smear was heat fixed by passing 2-3 times through a flame. Crystal violet stain was poured on the slide and

allowed to act for 1-2 minutes after which the slide was washed under running water. Excess water on the slide was tipped off. Gram's iodine was then applied for a minute, and washed off with water. Decolourisation of the slide was then done with an acetone-spirit solution for a few seconds and then washed off immediately with water. The slide was counter-stained with neutral red for 1-2 minutes, washed thoroughly with water and blotted dry. The stained, dried film was then finally examined under the oil immersion objective of a light microscope.

The Gram stained slide was scored for Bacterial vaginosis according to Nugent's criteria [304] (Table 3.1). With this criteria, a score is assigned on the basis of the proportions of bacterial morphological types seen, ranging from lactobacillus-predominant flora (score 0) to Gardnerella, Bacteroides and Mobilincus-predominant, lactobacillus-depleted flora (score 10). For each slide, scores are assigned depending on the number of each of the above individual morphotypes present; 0, no morphotype seen, 1, one morphotype seen, 2+, two to four morphotypes seen, 3+, five to thirty morphotypes seen, 4+, more than thirty morphotypes seen. A composite score of 7-10 from addition of the sub-totals is taken to be diagnostic of bacterial vaginosis, 4-6, an intermediate smear and 0-3, a normal smear.

Table 3.1 Nugent's criteria for Bacterial vaginosis scoring system (adapted from Nugent RP, Krohn MA, Hillier SL [304]).

Score	Lactobacillus Morphotype	Gardnerella & Bacteroides spp	Curved gram variable rods
0	4+	0	0
1	3+	1	1 or 2+
2	2+	2+	3+ or 4+
3	1	3+	
4	0	4+	

Any yeast cells and hyphae in the Gram stained smear were also noted

3.11.9 Storage of *Trichomonas vaginalis* isolates

This was carried out for all InPouch isolates of *T.vaginalis*. 1 ml of positive InPouch culture was put in a cryovial. To this was added 150 μl inactivated horse serum and 100 μl DMSO. The resulting solution was mixed very well, and incubated at -20°C for an hour and then transferred to a -70°C freezer thereafter for long term storage. All isolates were stored in duplicate, and subsequently shipped on dry ice to London.

3.12 Data Management

Data were pre-coded on the questionnaire and forms. Completeness of questionnaire responses and other forms was checked each day on-site at the hospitals. Data entry templates were created in Epi Info 6.03 (CDC, Atlanta) and data were double-entered by 2 clerks. Contradictory responses were identified using range and consistency checks in Epi Info. Validation of the double data entry was done at the end of each week by the Epi Info validate programme.

Data was transferred to and analysed in STATA 8 (STATA Corporation, Texas, US). The distribution of each exposure variable was examined separately for cases and

controls. Outliers were checked against original questionnaire and errors corrected before data analysis. Quantitative variables were grouped into known categories of the variable or into categories commonly used in the STI literature.

3.12.1 Data analysis

3.12.1.1 Case control study

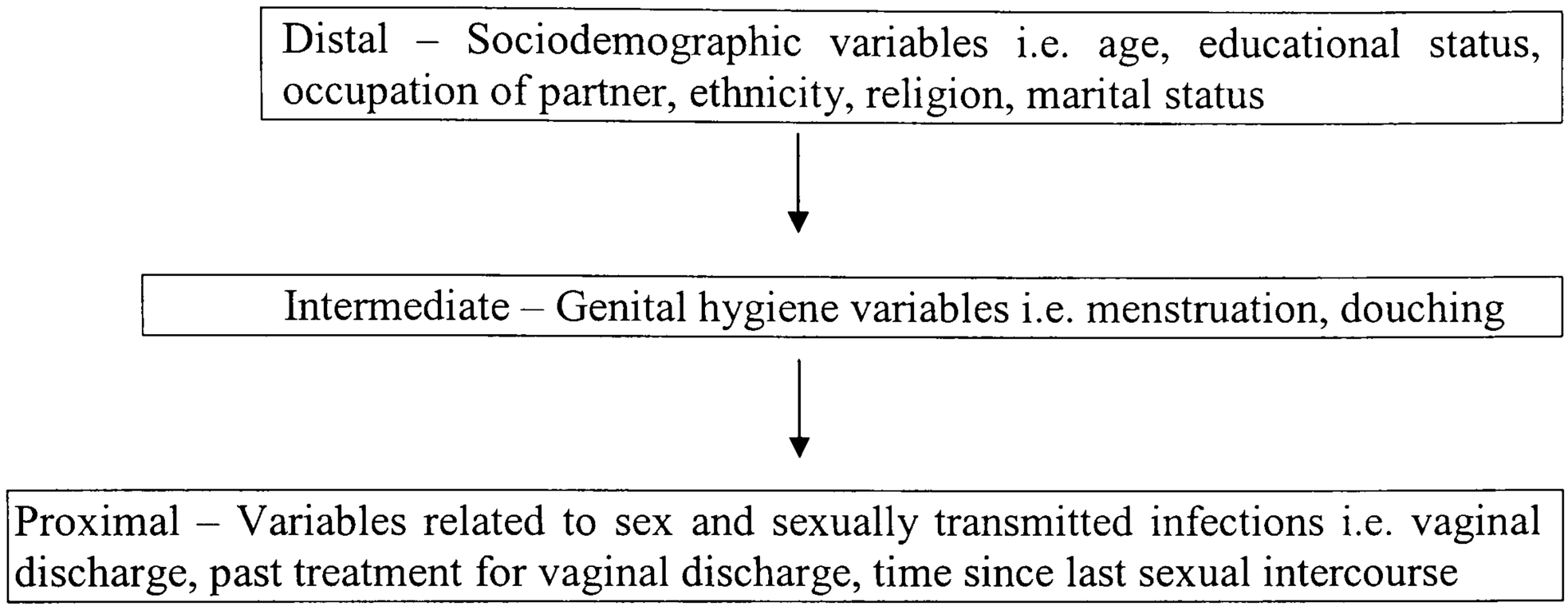
The data was analysed as an unmatched study. Unmatched analyses was done due to practical difficulties, as controls were the following two consecutive women attending the clinic after each case and were not matched on age or any other potential confounding factors. As these potential confounders could distort the size of any measure of effect, logistic regression methods were used to control for this.

Baseline variables were usually taken as the lowest quantile of exposure or group considered to be at lowest risk of *T.vaginalis* infection. Proportions were compared using the χ^2, and Fisher's exact tests. Using logistic regression, odds ratios (OR) and their 95% confidence intervals (95% CI) were estimated for exposure variables with a diagnosis of *T.vaginalis* as outcome. The statistical significance of associations between exposure and risk of *T.vaginalis* infection was determined using Likelihood Ratio Tests (LRT) of homogeneity and of trend.

Again, using multiple logistic regression, models were developed to examine factors associated with *T.vaginalis* infection on univariate analysis at p<=0.1. For the modelling strategy, a conceptual framework [305] was used to explore the inter-relationships of exposures thought to lie at different positions on the same causal pathway. Variables were categorised according to their proximity to the risk of *T.vaginalis* infection and were added in turn to the model starting with the distal

variables and ending with the proximal ones. Figure 3.6 illustrates what was done in this study.

Figure 3.4 Modelling strategy for variables independently associated with TV infection



First, the association between the socio-demographic factors and TV infection were investigated. Variables were kept in the model at each stage if they remained significantly associated with *T.vaginalis* infection at p<=0.1. Next, the association between each variable in the other categories and TV infection was assessed by adding the variable into the above model one by one in order to adjust for the socio-demographic variables. A composite multivariate model, consisting of all distal, intermediate, and proximal category variables associated with TV infection at p<=0.1 was formed. The final multivariate logistic regression model was reached by dropping factors in the composite model one at a time until all remaining factors were significantly associated with infection at p<=0.1 using the likelihood ratio test (LRT). This model estimated the independent effects of all variables on *T.vaginalis* infection.

An investigation of whether the factors independently associated with infection, either singly or in combination could be used as a tool to predict TV infection in the

study population was done. To do this, binary scores were developed for each factor independently predicting infection on multivariate analysis in the final model. Two approaches in developing the scores were followed. In the simple approach scores of 1 and 0, were given to each study subject, with the score of 1 for subjects with the factor and 0, those without. This system, it was believed, would be easy to apply in a field setting. This was compared to a second scoring system where weights were given to individual factors associated with infection in the final model depending on their odds ratios (OR); score 1 for OR between 1-2, 2 for OR between 2-3, score 3 for OR between 3-4 and score 5 for OR between 5-6, and 0 if no association existed. A combined score, the sum of all the factors independently associated with infection present in each subject was calculated using both systems

Using these scores as cut-offs, the diagnostic values (sensitivity, specificity, and predictive values) of various combinations of the factors in predicting *T.vaginalis* infection was calculated. As only the selected case-control cohort had been tested with the gold standard TV diagnostic test, an estimation of the number of women in the target population who would have tested positive on the gold standard (and hence those testing negative) was done. In doing this, it was assumed that women in the target population who were not selected for the case control study would not differ in terms of the gold standard tests from those selected.

Finally, the diagnostic values when the scoring systems of the socio-demographic and clinical parameters predicting infections were combined, was investigated.

3.12.1.2 Diagnostic comparison

Using a combination of wet prep and/or culture as expanded gold standard, the performance of the various diagnostic tests for TV (latex agglutination, wet prep,

culture, enzyme immunoassay, lateral flow and PCR) was done. Sensitivity, specificity, and predictive values of the tests in the target population were estimated. The Kappa index of test agreement between the individual tests and the expanded gold standard was calculated.

3.12.1.3 Are trichomonads other than T.vaginalis involved in the aetiology of vaginal trichomoniasis?

To answer whether trichomonads other than *T.vaginalis* are involved in the aetiology of vaginal trichomoniasis, a comparison was made between the results of traditional TV tests (wet prep and culture) and PCR results of vaginal swab eluates using specific primers for *P. hominis, T. tenax* and *T.vaginalis.*

3.12.1.4 Presence of trichomonads in various body sites

Frequencies were used to estimate the presence of trichomonads in various body sites. The trichomonads were identified from vaginal, rectal and oral swabs by PCR.

3.12.1.5 Clinical efficacy of single 2g metronidazole therapy in pregnant women

Frequencies of women testing positive for *T.vaginalis* at test of cures were compared with those testing negative.

4. RESULTS: DEMOGRAPHIC AND BEHAVIOURAL PREDICTORS OF VAGINAL TRICHOMONIASIS IN PREGNANT WOMEN

4.1 Study Population

A total of 3807 women consented to participate in the study. This included all those testing positive for TV by the latex agglutination test (LAT), and the randomly selected women testing negative.

All women meeting the inclusion/exclusion criteria agreed to participate in the study. However, there were 9 women who after initially obtaining their self administered vaginal swabs for latex agglutination testing (4 positives, 5 negatives) could not be traced for subsequent parts of the study i.e. interviewing, clinical examination and obtaining further vaginal, oral and rectal swabs. They were dropped and substituted.

4.2 Prevalence of TV infection

Of the 3807 women, 206 were positive on LAT testing, giving a prevalence of 5.4%. All the 206 women and 427 women negative on LAT were selected as the cohort for the case control study. Using the expanded gold standard of wet prep and/or culture (see 4.3), the prevalence of TV was 4.93% (188/3807) when estimated for the study target population.

4.3 Case and control group

The decision to use the LAT for screening was based on its reported sensitivity and specificity [8]. It was believed that the accuracy of the LAT would be good enough in selecting patients with trichomoniasis so that subsequent same day interviewing and

examination of the participants could be done, instead of recalling them after a week when gold standard test results were available. Many of them could then be lost to follow up.

Due to the fact that the LAT was being evaluated against an expanded gold standard (EGS) TV diagnostic and the performance of the LAT in this population was unknown, it was decided rather to use results of the EGS in defining cases and controls for the case control study. All the 633 women selected on the basis of the LAT result were also tested with the other TV diagnostics. Cases were thus defined by an expanded gold standard (EGS) as wet prep and/or culture positive. Controls were negative for both tests.

4.4 Univariate and age-adjusted analysis

4.4.1 Sociodemographic characteristics of cases/controls

4.4.1.1 Age

The age of participants ranged between 15 and 44 years, with a median age of 26 years. The cases were younger on average than the controls, and there was a strongly significant association between age and infection ($p=0.0001$). Subsequently, all analysis of the association between TV infection and all variables was age adjusted (Table 4.1).

Variable	Cases n(%)	Controls n(%)	Crude OR	95% CI	Age Adjusted OR	95% CI	p (age-adjusted, LRT)
Age (years)							#0.0001
>30	34 (19.7)	123 (26.8)	1				*0.005
25-29	47 (27.2)	153 (33.3)	1.11	0.6-1.8			**0.07
20-24	61 (35.3)	154 (33.5)	1.43	0.8-2.8			
<20	31 (17.9	30 (6.5)	3.73	2.0-7.0			
Educational status							*0.03 **0.06
None	27 (15.6)	62 (13.5)	1		1		
Primary	24 (13.9)	74 (16.1)	0.74	0.4-1.4	0.64	0.32-1.2	
Jun Sec	87(50.2)	264 (57.4)	0.75	0.4-1.2	0.73	0.43-1.2	
>Jun Sec	35 (20.2)	60 (13)	1.33	0.7-1.3	1.45	0.77-2.7	
Occupation							0.4
Trader	75 (43.3)	230 (50)	1		1		
Housewife	30 (17.3)	67 (14.6)	1.37	0.8-2.3	1.31	0.8-2.2	
Professional	56 (32.4)	132 (28.7)	1.30	0.8-1.9	1.20	0.7-1.8	
Student	9 (5.2)	24 (5.2)	1.15	0.5-2.6	0.54	0.2-1.3	
Farmer	3 (1.8)	7 (1.52)	1.31	0.3-5.2	1.53	0.4-6.1	
Religion							0.08
Christian	142 (82)	379 (82.4)	1		1		
Moslem	25 (14.4)	77 (16.7)	0.86	0.53-1.4	0.78	0.5-1.3	
No religion	6 (3.5)	4 (0.9)	4.00	1.1-14.4	3.58	1.0-13.0	
Ethnicity							0.005
Akan	125 (72.2)	356 (77.4)	1		1		
Northerner	29 (16.8)	85 (18.5)	0.97	0.6-1.5	0.81	0.5-1.3	
Other	19 (11)	19 (4.1)	2.84	1.4-5.5	2.89	1.4-5.6	
Marital status							0.03
Monogamous	143(83.6)	418 (90.9)	1		1		
Polygamous	15 (8.7)	34 (7.4)	1.28	0.7-2.4	1.42	0.7-2.7	
Single	15 (8.7)	8 (1.7)	5.48	2.2-13.1	3.18	1.2-8.4	
Age at first marriage (years)							1.0
<20	60 (34.7)	132 (28.7)	1		1		
>=20	113(65.3)	328 (71.3)	0.75	0.5-1.1	1	0.67-1.5	

Table 4.1. Association between Socio-demographic variables and TV Infection. Likelihood ratio test (LRT), # unadjusted, p value for trend *categorical and **linear models.

4.4.1.2 Educational status

Number of years of formal schooling was comparable among cases and controls. 15.6% and 13.5% of cases and controls respectively had no formal education. Whilst 13.9% of cases had been up to the basic primary level (6 years schooling) only, 16.1% controls had similar attainment. 50.2% cases and 57.4% controls had attained up to the Junior secondary school (9 years schooling). 20.2% cases and 13% controls had some education after Junior secondary level. This included those attaining senior secondary and tertiary education levels.

In general, education was significantly associated with infection (p=0.03). For those having some formal education, infection was associated with increasing number of years schooling at significant levels (p=0.03).

4.4.1.3 Occupation

Occupations of the women illustrate the Kumasi metropolis as the hub of trading activities in the region, country and the West Africa sub-region. The most common occupation of the women was trading; 43.3% cases and 50% controls. Housewives made up 17.3% and 14.6% of the population of cases and controls respectively, whilst an equal proportion (5.2%) of both cases and controls were students. Hairdressers, dressmakers and their apprentices, office assistants, classified as professionals, represented 32.4% cases and 28.7% controls. There is hardly any farming activity in the metropolis, residents relying on food from nearby villages. Farmers among the women were 1.8% of cases and 1.5% of controls. There was no association between infection and occupation (p=0.4)

4.4.1.4 Religion

Christianity was the professed faith among 82% cases and 82.4% controls, whilst the Islamic faith was represented by 14.4% and 16.7% of cases and controls respectively. 3.5% cases and 0.9% controls said they had no religious faith. The odds ratio for infection of having no religious faith was 3.58. Religion was associated with infection at borderline significance (p=0.08)

4.4.1.5 Ethnicity

As expected, 72.2% of cases and 77.4% controls were Akans. 16.8% cases and 18.5% controls were Northerners. Other ethnic groups represented 11% and 4.1% cases and controls respectively. The odds ratio for infection of being neither Akan nor Northerner was 2.89 (95% CI 1.4-5.6). Ethnicity was significantly associated with infection at p=0.005

4.4.1.6 Marital status

The majority of women were in monogamous relationships (83.6% cases, 90.9% controls). 8.7% and 7.4% of the women had polygamous partners. There were more single women among the cases (8.7%) than the controls (1.7%). The odds ratio for infection of being single was 3.18. Marital status was significantly associated with infection (p=0.03).

4.4.1.7 Age at first marriage

Whilst 34.7% of cases were married before the age of 20 years, this was the case for 28.7% of controls. Amongst those being married at age 20 years or more, 65.3%

were cases and 71.3% controls. There was no association between age at first marriage and TV infection (p=1.0).

In summary, for the socio-demographic factors studied, age, education, marital status, and ethnicity were significantly associated with infection. Religion was of borderline significance.

4.4.2 Maternal factors

Table 4.2 shows the associations between maternal factors and TV infection.

Table 4.2. Association between maternal factors and TV infection

Variable	Cases n(%)	Controls n(%)	Crude OR	95% CI	Age Adjusted OR	95% CI	p (Age adjusted LRT)
Gestation Stage (Trimesters)							0.85
First	32 (18.5)	91 (19.8)	1		1		
Second	81 (46.9)	206 (44.8)	1.18	0.7-1.8	1.14	0.7-1.9	
Third	60 (34.6)	163 (35.4)	1.04	0.6-1.7	1.08	0.65-1.8	
Circumcision							0.82
No	169 (97.7)	446 (97.0)	1		1		
Yes	4 (2.3)	14 (3.0)	0.75	0.2-2.3	0.88	0.3-2.7	

4.4.2.1 Stage of gestation

18.5% of cases as against 19.8% controls were in their first trimester of pregnancy while 46.9 cases and 44.8% controls were in the second trimester. Study subjects in the third trimester comprised 34.6% cases and 35.4% controls. In Ghana most pregnant women start attendances at antenatal clinics from the second trimester. Gestation stage was not associated with infection.

4.4.2.2 Circumcision

2.3% cases as against 3% controls said they had been circumcised. All those circumcised did not know why they were circumcised as it was done when they were children. Age at circumcision for both cases and controls ranged 2 years – 14 years, with a mean age of 6.2 years for cases and 7.3 years for controls. Circumcision was not associated with infection.

None of the maternal variables studied were significantly associated with infection (Table 4.2).

4.4.3 Partner related factors

Table 4.3. Association between partner related factors and TV infection

Variable	Cases n(%)	Controls n(%)	Crude OR	95% CI	Age Adjusted OR	95% CI	p (Age adjusted, LRT)
Partners' occupation							0.45
Non-professional	145 (83.8)	391 (85)	1		1		
Professional	28 16.2)	69 (15)	1.09	0.7-1.7	1.20	0.73-1.1	
Partner ever treated for STI							0.17
No/don't know	168 (97.1)	454 (98.7)	1		1		
Yes	5 (2.9)	6 (1.3)	2.25	0.7-7.5	2.40	0.7-8.1	
Partner having extra marital sex							0.42
No	129 (74.6)	352 (76.5)	1		1		
Yes	44 (25.4)	108 (23.5)	1.11	0.7-1.6	1.13	0.7-1.7	

4.4.3.1 Partners' occupation

83.8% of partners of cases as against 85% controls were non-professionals. This included students, farmers, traders, artisans, drivers and the unemployed. Professionals, who included teachers, bankers, and lawyers, formed 16.2% and 15% of cases and controls respectively. Partners' occupation was not significantly associated with infection.

4.4.3.2 Past treatment for sexually transmitted infections (STI)

Only 2.9% of cases and 1.3% of controls said they knew their partners had ever been treated for a sexually transmitted infection. The rest did not know whether this was the case . Past treatment for STI was not significantly associated with infection (p=0.17)

4.4.3.3 Extra marital sexual activities of partner

25.4% of cases and 23.5% of controls said their partners were cheating on them. This difference was not statistically significant (p=0.4).

Again, none of the partner related factors were significantly associated with infection (Table 4.3). Even though the OR of a partner ever being treated for STI was 2.4 (age adjusted), this was not statistically significant (p=0.17).

4.4.4 Anogenital hygiene practices (Table 4.4).

Devices used by the women to protect menstrual flow were; sanitary pad (46.2% cases, 37.1% controls), toilet roll (24.9% cases, 34.4% controls), clean washed folded cloth (21.9% cases, 24.4% controls) and cotton wool (7% cases, 4.1% controls). 52.6% and 54.7% of cases and controls respectively changed whatever

device they used twice in the day. While 44.5% cases and 43.5% controls changed more than twice in the day, 2.9% cases and 1.8% controls changed their devices only once in the day. Whilst the use of toilet roll compared to the commonly used sanitary pad seemed protective of infection (OR=0.58, 95% CI 0.4-0.9), the use of cotton wool was associated with infection (OR=1.64, 95% CI 0.75-3.6) (Table 4.4). There was no significant association between how often the "devices" were changed and infection (p=0.7).

After defecation, the women used toilet roll, paper other than toilet roll (newspaper mainly) or other device (dry corn cob, hand and water) to clean up. Toilet roll was used by 36.4% cases and 33.7% controls, other paper by 57.2% cases and 58.3% controls and other device by 6.4% and 8% cases and controls respectively. The use of any of these "sanitary aids" at defecation was not significantly associated with infection (p=0.4).

80.3% of cases and 82.4% controls cleaned themselves from front to back (away from the vagina) after defecating. While cleaning from back towards the front (towards vagina) was done by 17.9% and 15.2% cases and controls respectively, cleaning in either direction was done by 1.8% of cases and 2.4% controls. The direction of cleaning was not significantly associated with infection (p=0.8)

39.9% of cases and 45.7% controls did not wipe their genitalia after urinating. At p=0.21, wiping the genitalia after urinating was not significantly associated with infection

Douching was practised by 13.3% of cases and 7.8% controls. Reasons for douching were to clear a vaginal discharge (47.8% cases, 73% controls), to feel clean generally (39.1% cases, 21.6% controls) and feel clean after menstruation and/or sexual

intercourse (13% cases, 5.4% controls). This practice was significantly associated with infection (OR=1.94, 95%CI 1.1-3.4, p=0.02)

Table 4.4. Association between ano-genital hygiene practices and TV infection

Variable	Cases n (%)	Controls n (%)	Crude OR	95% CI	Age Adjusted OR	95% CI	p (Age adjusted ,LRT)
Menstruation "device"							0.02
Sanitary pad	80 (46.2)	171 (37.1)	1		1		
Toilet roll	43 (24.9)	158 (34.4)	0.58	0.4-0.9	0.58	0.4-0.9	
Folded cloth	38 (21.9)	112 (24.4)	0.72	0.5-1.1	0.77	0.5-1.2	
Cotton wool	12 (7.0)	19 (4.1)	1.35	0.6-3.0	1.64	0.75-3.6	
Number of times "device" changed daily							0.70
Once	5 (2.9)	9 (1.8)	1.53	0.5-4.7	1.62	0.52-5.05	
More than twice	77 (44.5)	200 (43.5)	1.06	0.74-1.5	1.05	0.73-1.5	
Twice	91 (52.6)	251 (54.7)	1		1		
Defecation hygiene							0.4
Toilet roll	63 (36.4)	155 (33.7)	1		1		
Paper other than toilet roll	99 57.2)	268 (58.3)	0.90	0.62-1.3	0.79	0.5-1.1	
Other	11 (6.4)	37 (8.0)	0.73	0.35-1.5	0.69	0.3-1.5	
Direction of cleaning at defecation							0.8
Front to back	139 (80.3)	379 (82.4)	1		1		
Back to front	31 17.9)	70 (15.2)	1.20	0.7-1.9	1.10		
Either	3 (1.8)	11 (2.4)	0.74	0.2-2.7	0.72		

Wiping after micturition							0.21
No	104 (60.1)	250 (54.3)	1		1		
Yes	69 (39.9)	210 (45.7)	0.78	0.5-1.1	0.79	0.5-1.1	
Douche							0.02
No	150 (86.7)	424 (92.2)	1		1		
Yes	23 (13.3)	36 (7.8)	1.80	1.0-3.1	1.94	1.1-3.4	

4.4.5 Factors relating to sex and sexually transmitted infections (STI)

Age at initiation of sexual intercourse ranged 12-31 years with a median of 18 years. There was no association between age at initiation of sexual intercourse and infection.

Table 4.5. Association between variables relating to sex and STI and TV infection.

Variable	Cases n(%)	Controls n(%)	Crude OR	95% CI	Age Adjusted OR	95% CI	p (Age adjusted, LRT)
Age at first sexual intercourse (years)							0.93
<19	150 (86.7)	410(89.1)	1		1		
>=19	23 (13.3)	50 (10.9)	1.25	0.74-2.1	0.97	0.5-1.7	
Time since last sexual intercourse							0.005
Within 2 weeks	93 (53.8)	308 (66.9)	1		1		
Over two weeks	80 (46.2)	152 (33.1)	1.74	1.2-2.5	1.68	1.1-2.4	
Vaginal discharge symptom							0.001
No	71 (41)	262 (57)	1		1		
Yes	102 (59)	198 (43)	1.90	1.3-2.7	1.80	1.2-2.6	
Vuval itch							0.93
No	119 (68.8)	326 (70.9)	1		1	0.66-1.45	
Yes	54 (31.2)	134 (29.1)	1.10	0.7-1.6	0.98		
Dysuria							0.4
No	148 (85.5)	388 (84.4)	1		1		
Yes	25 (14.5)	72 (15.6)	0.91	0.55-1.5	0.83	0.5-1.3	
Past treatment for vaginal discharge							0.006
No	147 (85)	425 (92.4)	1		1		
Yes	26 (15)	35 (7.6)	2.10	1.25-3.7	2.20	1.2-3.8	
Genital ulcer symptom							0.5
No	152 (87.9)	398 (86.5)	1	129	1		

53.8% and 66.9% of cases and controls respectively had had their last intercourse within 2 weeks of being enrolled into the study. The rest had had their last sexual intercourse more than 2 weeks before interview.

Significantly more cases (59%) than controls (43%) had symptomatic vaginal discharge on questioning (p=0.001). While 31.2% of cases complained of a vulval itch, this was so for 29.1% of controls. Symptomatic genital ulceration was alluded to by 12.1% and 13.5% cases and controls respectively. Dysuria was complained of by 14.5% cases and 15.6% controls. On past treatments for vaginal discharge, 15% cases as against 7.6% controls admitted such treatments.

Table 4.5 shows the associations between infection and variables related to sex and sexually transmitted infections. A presenting symptom of vaginal discharge was strongly associated with infection (OR 1.80, 95%CI 1.2-2.6, p=0.001), as was past treatment for vaginal discharge (OR 2.20, p=0.006). Women whose last time of having intercourse was more than 2 weeks before being seen at the clinic, were significantly more likely to have TV infection than those who had had sex within 2 weeks of being seen (OR 1.68, p=0.005). Age at initiation of sexual intercourse, and STI related symptoms such as genital itch, and genital ulcer were not significantly associated with TV infection.

4.4.5.1 Sexual practices

Questions on sexual practices included oral and anal sex from partners and the practice of women lubricating their vaginas with saliva prior to penetration due to vaginal dryness. 18.6% of cases and 16.1% controls reported receiving oral sex from their partners. 4% of cases and 5.7% controls had anal intercourse with their partners. Due to problems of vagina dryness, 11.6% and 13.5% of cases and controls

respectively lubricated their vagina with saliva either themselves or from their partners.

Both oral and anal sex from the participants' partners were not significantly associated with infection, just as was whether or not they lubricated their genitalia with saliva prior to intercourse (Table 4.6).

Table 4.6 Association between sexual practices and TV infection.

Variable	Cases n(%)	Controls n(%)	Crude OR	95% CI	Age Adjusted OR	95% CI	p (Age adjusted ,LRT)
Lubricating vagina with saliva during sexual intercourse							0.67
No	15(88.4)	398 (86.5)	1		1		
Yes	20(11.6)	62 (13.5)	0.83	0.5-1.4	0.89	0.5-1.5	
Oral sex from partner							0.43
No	142 (82)	386 (83.9)	1		1		
Yes	31(18.6)	74 (16.1)	1.13	0.7-1.8	1.20	0.7-1.9	
Anal sex with partner							0.43
No	166 (96)	434 (94.3)	1		1		
Yes	7 (4)	26 (5.7)	0.70	0.3-1.6	0.71	0.3-1.7	

4.5 Multivariate analysis

To determine which of these factors best predicted women most likely to have TV infection, a logistic regression analysis allowing for adjustment of all factors associated with infection at p<=0.1 was performed in 2 stages. First a composite model was built by fitting variables from all categories. A final model was then fitted by adjusting for all variables in the composite model.

Socio-demographic variables selected for multivariate analysis were; age, educational status, ethnicity, marital status and religion. "Devices" used to protect

menstrual flow and douching were the ano-genital hygiene variables selected. Factors relating to sex and sexually transmitted infections, including self report of symptomatic vaginal discharge, past treatment for a vaginal discharge and time since last sexual intercourse were also put in the multivariate model. None of the variables studied under partner related factors, maternal factors and sexual practices attained significance at p<=0.1 when adjusted for age and so were not included in model (Table 4.7).

Table 4.7. Multivariate analysis of factors predictive of TV infection

Variable	Adjusted OR*	95% CI	P (LRT)
Age (years)			0.005
>=30	1		
25-29	1.09	0.6-1.8	
20-24	1.47	0.8-2.4	
<20	3.45	1.6-7.1	
Educational status			0.04
None	1		
Primary	0.59	0.3-1.2	
Junior secondary	0.71	0.4-1.3	
>Junior secondary	1.35	0.7-2.7	
Ethnicity			0.007
Akan	1		
Northerner	1.04	0.5-2.0	
Other	3.13	1.5-6.0	
Marital status			0.04
Monogamous	1		
Polygamous	1.50	0.7-3.0	
Single	3.02	1.1-8.1	
Religion			0.08
Christian	1		
Moslem	0.66	0.35-1.2	
No religion	3.36	0.8-13.5	
"Device" for menstruation			0.04
Sanitary pad	1.00		
Toilet roll	0.57	0.4-0.9	
Folded cloth	0.80	0.5-1.4	
Cotton wool	1.50	0.7-3.4	
Douche			0.02
No	1.0		
Yes	2.0	1.1-3.6	

Symptomatic vaginal discharge No Yes	 1 1.78	 1.2-2.6	0.003
Past treatment for vaginal discharge No Yes	 1 1.63	 0.9-3.0	0.12
Time since last sexual intercourse Within 2 weeks Over 2 weeks	 1 1.71	 1.1-2.5	0.007

* adjusted for all variables in table

In multivariate analysis (Table 4.7), young age i.e. less than 20 years, neither being Akan nor Northerner (the predominant ethnic groups in Ashanti) (OR=3.0, 95%CI=1.5-6.5), and being single (OR=3.0, 9% CI=1.1-8.1) were independently associated with infection. Having no religious faith was also independently associated with infection at borderline significance (OR=3.36, 95% CI=0.8-13.5, p=0.08).

When adjusted for other factors, the use of cotton wool appeared independently associated with infection (OR=1.50), whilst using toilet roll was protective of infection (OR=0.57).

Even though educational status was associated with infection (p=0.04), there was no overall trend in the association.

Also independently associated with infection were douching, symptomatic vaginal discharge, and if time between examination and last sexual intercourse was more than 2 weeks. Past treatment for vaginal discharge was not independently associated with infection on multivariate analysis (OR 1.6, CI=0.9-3.0, p=0.12). This was excluded from the model.

Thus, the composite multivariate model covering all factors studied comprised the following factors; age, educational status, ethnicity, marital status, religion, sanitary

device used during menstruation, douching, complaining of a vaginal discharge, and having had sexual intercourse 2 or more weeks prior to being seen by study investigators.

4.5.1 Final multiple logistic regression model

The final model was reached after adjusting for all variables associated with infection at p<=0.1 in the compodsite model, and removing factors no longer statistically significant (Table 4.8);

Table 4.8. Final multiple logistic regression model of factors independently associated with TV infection (* adjusted for all variables in final model)

Factor	*Adjusted OR	95% CI	p(LRT)
Age			0.001
>=30	1		
25-29	1.04	0.6-1.7	
20-24	1.33	0.8-2.2	
< 20 years	3.36	1.5-7.1	
Ethnicity			
Akan	1		0.008
Northerner	0.92	0.4-1.7	
Other	3.18	1.4-6.8	
Religion			0.03
Christian	1		
Moslem	0.61	0.31-1.18	
No religion	4.45	1.0-19.1	
"Device" for menstruation			0.02
Sanitary pad	1		
Toilet roll	0.53	0.3-8.5	
Folded cloth	0.76	0.4-1.3	
Cotton wool	1.53	0.6-3.5	
Douche			0.05
No	1		
Yes	1.84	1.0-3.37	
Vaginal discharge symptom			0.003
No	1		
Yes	1.78	1.2-2.6	

Last sexual intercourse 2 or more weeks ago			0.007
No	1		
Yes	1.71	1.1-2.5	

Being single (p=0.17) was dropped because it was no longer significantly associated with infection. Educational status was also dropped (p=0.12)

4.6 Deriving binary variables from factors independently predicting infection

The possibility of using the variables independently associated with infection either singly or in combination in an algorithm fashion to predict TV infection in the study population was explored. To this end, binary variables for all the factors were derived, and their odds ratios for infection calculated. For devices used during menstruation, all devices except toilet roll use were deemed positively associated with infection. The use of toilet roll was protective of infection. Results are shown in Table 4.9.

Table 4.9. Odds ratios and 95% CI of generated binary variables predictive of TV infection

Factor	Adjusted Odds ratio*	95% CI
Age <20 years	3.10	1.7-5.5
Ethnicity other than Akan or northerner	3.05	1.5-6.0
No religion	4.97	1.2-19.8
Douche	1.78	1.0-3.2
No toilet roll use at menstruation	1.66	1.1-2.5
Vaginal discharge symptom	1.87	1.3-2.7
Last sexual intercourse 2 or more weeks ago	1.75	1.2-2.5

* adjusted for all other binary factors

4.7 Diagnostic values of factors independently associated with infection

The sensitivity, specificity and predictive values of the factors independently predicting TV infection were calculated for the target population. As seen in Table 4.10, sensitivities ranged between 3.7% -75.5%, and positive predictive values 5.6-18.4%. The highest sensitivity, 75.5%, was shown by the non-use of toilet roll for menstrual hygiene. This also had the least specificity (34.3%)

Table 4.10. Diagnostic values of factors independently associated with TV infection

Factor	**Sensitivity (%)**	**Specificity (%)**	**Positive predictive value (%) in target population**	**Negative predictive value (%) in target population**
Age < 20 years	18.0	93.5	12.5	94.7
Ethnicity other than Akan or Northerner	11.2	95.8	12.3	95.4
No religion	3.7	99.1	18.4	95.1
No toilet roll use at menstruation	75.5	34.3	5.6	96.4
Douche	13.2	92.1	8.1	95.3
Vaginal discharge symptom	59.0	56.9	6.6	96.3
Last sexual intercourse 2 or more weeks ago	46.3	66.9	6.8	96.0

4.8 Binary scoring

Two scoring systems - simplified and weighted systems, were developed.

4.8.1 Simplified scoring system

Sores of 1 and 0 were assigned to each of the 633 women depending on whether or not they had any of the factors independently associated with TV infection, with 1 for having the factor and 0 if it was absent, and the total score per subject calculated. A score of 1 for a subject meant they had only 1 of being of a young age, neither being Akan nor northerner, of no professed faith, not using toilet roll to protect menstrual flow, practicing douching, having sexual intercourse more than 2 weeks before being seen, and complaining of a vaginal discharge. A score of 2 indicates they had 2 factors etc. The maximum possible score with this system is 7.

Distribution of the simple scoring system among cases and controls is shown in Table 4.11. Whilst the lower end of the scoring system (0-1) predominantly comprised controls, cases predominantly made up scores 3-6. Score 2 appeared evenly distributed amongst cases and controls.

Table 4.11. Distribution of scores among cases and controls

Score	**Cases (%)**	**Controls (%)**	**Total**
0	4 (2.3)	53 (11.5)	57
1	35 (20.2)	163 (35.4)	198
2	63 (36.4)	165 (35.9)	228
3	56 (32.4)	68 (14.8)	124
4	14 (8.1)	11 (2.4)	25
6	1 (0.6)	0 (0)	1
Total	173 (100)	460 (100)	633

4.8.2 Weighted scoring system

Weights given to variables independently associated with infection depended on their odds ratios for TV infection; 1 if OR =1-2, 2 if OR =2-3, 3 if OR = 3-4, and 4 if OR = 4-5. These are as listed in Table 4.12.

Table 4.12. Weighted scores depending on Odds ratios

Factor	**Adjusted OR**	**Weighted score**
Age < 20 years	3.10	3
Ethnicity other than Akan or Northerner	3.05	3
No religion	4.97	4
Douche	1.78	1
No toilet roll use at menstruation	1.66	1
Vaginal discharge symptom	1.87	1
Last sexual intercourse 2 or more weeks ago	1.75	1

A maximum 14 scores is obtainable by a subject with this system. Table 4.13 shows the distribution of weighted scores among cases and controls

Table 4.13. Distribution of weighted scores among cases and controls

Score	**Cases (%)**	**Controls (%)**	**Total**
0	4 (2.3)	53 (11.5)	57
1	32 (18.5)	159 (34.6)	191
2	53 (30.6)	146 (31.7)	199
3	30 (17.3)	49 (10.6)	79
4	13 (7.5)	22 (4.8)	35
5	28 (16.2)	22 (4.8)	50
6	10 (5.8)	8 (1.7)	18
7	1 (0.6)	1 (0.2)	2
9	1 (0.6)	0 (0)	1
13	1 (0.6)	0 (0)	1
Total	173 (100)	460 (100)	633

Mirroring the simple scoring system is the predominance of controls among the lower scores (0-2). 9% of the 633 participants did not register any score.

4.9 Diagnostic value of scoring systems in study population

Using each score as a cut-off point, the sensitivity, specificity and predictive value of a positive score for the selection of a woman with *T. vaginalis* infection was determined (Table 4.14). The percentage of women in the study population who would have been selected for screening and/or treatment identified by each cut-off score was also calculated.

Table 4.14. Diagnostic value of simplified scoring system

Cut-off score	**Sensitivity (%)**	**Specificity (%)**	**Positive predictive value (%) in target population**	**Negative predictive value (%) in target population**	**Women in study population selected n=3807 (%)**
> = 1	97.7	11.5	5.4	99.0	3386 (88.9)
> = 2	77.6	46.9	7.0	97.6	2066 (54.3)
> = 3	40.9	82.8	11.0	96.4	699 (18.3)
> = 4	8.5	97.5	15.5	95.3	103 (2.7)
6	0.5	100	100	95.0	1 (0.03)

Sensitivity of the cut-off scores in the simplified system for the diagnosis of TV infection decreased with increasing scores. The proportion of subjects with various combined scores who would have *Trichomonas vaginalis* infection as per the gold standard test (positive predictive value) increased with increasing combined scores. The highest positive predictive value was attained when subjects had a score of 6. This would have meant a selection of only 1 out of 3807 women in the population for treatment or screening. The combination with the greatest sensitivity (97.7%) had a

positive predictive value of only 5.4%. With this cut-off, up to 89% of the study women would be selected for screening/treatment.

As in the simplified system, the sensitivity of each cut-off point for TV diagnosis decreased with increasing weighted scores (Table 4.15). The positive predictive values increased likewise. The highest PPV achieved with higher scores selected only up to 2 subjects for the desired objectives. In similar vein to the simplified system, the combination with the greatest sensitivity (score >=1) had the lowest PPV (5.4%) and selected the majority of women (88.9%).

Table 4.15. Diagnostic value of weighted scoring system

Cut-off score	**Sensitivity (%)**	**Specificity (%)**	**Positive predictive value (%) in target population**	**Negative predictive value (%) in target population**	**Women in study population selected n=3807(%)**
>=1	97.7	11.5	5.4	98.8	3386 (88.9)
>=2	79.2	46.0	7.0	97.7	2100 (55.1)
>=3	53.2	77.8	11.0	97.0	903 (23.7)
>=4	31.3	88.4	12.3	96.1	476 (12.5)
>=5	23.9	93.2	15.6	95.9	289 (7.6)
>=6	7.4	98.0	16.5	95.3	85 (2.2)
>=7	1.6	99.7	27.2	95.1	11 (0.3)
>=9	1.0	100	100	95.1	2 (0.05)
13	0.5	100	100	95.0	1 (0.03)

For both scoring systems, the combination of variables independently associated with infection was good at ruling in infection with the higher scores (4-6 simplified system, 5-13 weighted system) but poor at ruling out infection, as expected in this low TV prevalent population.

Distribution of scores among TV symptomatic and TV asymptomatic subjects in the case-control cohort was examined (Table 4.16 and Table 4.17). Within each score band (for those registering 2 or more scores in the simplified system and score 2-6 in

weighted system), there were significantly more symptomatics than asymptomatics ($p<0.0001$). Asymptomatic cases represented the majority registering 1 score or less. Thus, patients were more likely to be symptomatic for TV infection if they had a combined score of 2 or more and asymptomatic if their combined score was less than 2.

Table 4.16. Score distribution among case-control women symptomatic and asymptomatic for TV infection (with simplified scoring system)

Score	Symptomatic n (%)	Asymptomatic n(%)	Total
0	3 (5.3)	54 (94.7)	57 (100)
1	65 (32.8)	133 (67.2)	198 (100)
2	136 (59.6)	92 (40.4)	228 (100)
3	109 (87.9)	15 (12.1)	124 (100)
4	24 (96.0)	1 (4.0)	25 (100)
6	1 (100)	0 (0)	1 (100)
Total	338	295	633

Table 4.17. Score distribution among case-control women symptomatic and asymptomatic for TV infection (with weighted scoring system)

Score	Symptomatic n (%)	Asymptomatic n(%)	Total
0	3 (5.2)	54 (94.8)	57 (100)
1	64 (33.5)	127 (66.5)	191 (100)
2	129 (64.8)	70 (35.2)	199 (100)
3	72 (91.9)	7 (8.9)	79 (100)
4	17 (48.6)	18 (51.4)	35 (100)
5	35 (70.0)	15 (30.0)	50 (100)
6	15 (83.3)	3 (16.7)	18 (100)
7	1 (50.0)	1 (50.0)	2 (100)
9	1 (100)	0 (0)	1 (100)
13	1 (100)	0 (0)	1 (100)
Total	338	295	633

For scores with observations, the odds ratio of being symptomatic increased with increasing score for both the simplified (OR 3.92, 95% CI 3.0-4.9, $p<0.001$) and weighted systems (OR 1.75, 95%CI 1.5-2.0, $p<0.001$).

4.10 DISCUSSION: DEMOGRAPHIC AND BEHAVIOURAL PREDICTORS OF INFECTION IN PREGNANT WOMEN

Reported prevalence rates of *T.vaginalis* infection in pregnancy varies between 3-48% [306, 307, 308]. The wide range could be due to differences in study population, geographic areas and importantly, the diagnostic criteria used. The 4.9% prevalence obtained in this study by the expanded gold standard test mirrors closely that found in the neighbouring West African country, Benin, where the prevalence rate in women in the general population is 3.2% by TV culture test. In general, prevalence rates of all STIs in West Africa are much lower than found in Eastern and Southern Africa.

It is estimated that 25-50% of women positive for *T.vaginalis* do not have any symptoms [101, 105, 110]. The presence of infection is usually established in many centres by the saline wet prep method, a test which detects infection only in approximately 65% of infected women [196, 197]. Culture, the most sensitive diagnostic test for TV infection is not cost effective as a screening procedure. It would therefore be of benefit to identify factors that could predict women at greatest risk of *T.vaginalis* infection. Such factors would be particularly useful in identifying subgroups of women to screen with the most sensitive test, and in settings where no laboratory testing can be done, in treating women most likely to have infection.

The objective of this part of the study was to determine such sociodemographic, maternal, and partner related factors associated with TV infection. It was also to determine the association between infection and factors related to ano-genital hygiene, sexual practices and sex and sexually transmitted infections. These factors

would aid the selection of subjects either for screening for infection or treatment of infection.

Key findings were the association of certain factors independently with infection. These were; young age (less than 20 years), being of neither Akan nor Northern ethnicity, and not of the Christian or Moslem faith. Douching, having a vaginal discharge and not having had sexual intercourse recently were also independently associated with infection. A complex relationship was observed for devices used to clean up menstrual flow; compared to using the sanitary pad (commonest device used by study population), the use of cotton wool was associated with infection, while using toilet roll was protective of infection. This is difficult to explain.

In many studies on the epidemiology of female trichomoniasis, one demographic factor that has differed from that observed in other sexually transmitted infections has been age. Trichomoniasis has been associated with increasing age, unlike gonorrhoea and Chlamydia infection [65, 116, 309]. This present study however found an association between younger age and infection, and concurs with that found in the four city study, where trichomonas infection decreased with increasing age in all cities except Yaoundé [10]. Young age (<20 years) in Ndola, Zambia remained independently associated with infection on multivariate analysis.

Unlike in Ndola where there was an inverse relationship between TV infection and increasing years spent in formal education (also seen in Zimbabwe [116]), this association was not very clear in the Kumasi population where the odds ratio of infection seemed to increase for those being educated for 12 or more years compared to no education, and decrease for those educated less than 12 years compared to the uneducated. Education in Ghana for more than 12 years implies some amount of tertiary education. It is possible that such women could have been married to men

with the same kind of education or even better. With more earning power, these men could have multiple partners from whom they acquire STIs and subsequently pass this on to their spouses. When adjusted for other variables independently associated with infection, educational status of the Kumasi women was not significantly associated with infection (p=0.12). In consonance with other sexually transmitted infections, women not married were at increased risk of TV infection [315]. In this population, this did not reach statistically significant levels when controlled for factors independently predicting infection.

Can religion with its moral teachings protect against acquisition of sexually transmitted infections? In this study, not professing any faith was associated with over 3 times the odds of infection and was also predictive of infection, reaching statistical significance (p=0.003) when controlled for other factors independent of infection. However the numbers involved are small.

It has been suggested that ano-genital hygiene could be associated with the risk of TV infection [100]. In attempting to look at the role of menstrual hygiene in this respect, the use of various devices in protecting menstrual flow was examined. A complex relationship ensued. The use of toilet roll and clean cloth was protective of infection (OR=0.6 and 0.8 respectively) compared to the sanitary pad, whilst using cotton wool was associated with infection (OR=1.5), all at a statistically significant level (p=0.04). This is difficult to explain.

The practice of douching has been reported to be associated with HIV infection and other STDs in many studies [310, 311, 312,]. Myer *et al* reported recently from South Africa that women who douched were significantly more likely to show evidence of trichomoniasis than those not douching (p=0.03) [313] . In Ghana, the practice of douching generally refers to the use of any substance within the vagina either by

insertion or washing. Such substance can include water alone, water with added chemicals, soap, herbs, or chemicals in rock form eg saltpetre. In this Kumasi study, douching was significantly associated with infection (OR=2.0, CI=1.1-3.6, p=0.02), and was an independent predictor of infection. Douching in Ghana is practiced for several reasons including for general genital hygiene. There have been anecdotal reports that in situations when abrasive herbs and detergents have been used, a detrimental effect on the health of the woman has ensued. These have included vaginal stenosis and the introduction of infection into the vagina. In recent times health education messages given to women have stressed the self cleansing nature of the vagina and the risks in douching. It is thought that as a practice, douching is losing its proponents and younger women are abandoning the practice. Further research on the practice and its effects on reproductive health would better clarify the situation.

Also for poor ano-genital hygiene, the study looked at the possible role of the direction of cleaning the ano-genital area after defecation. It was hypothesised that if rectal trichomonads were involved in the aetiology of vaginal trichomoniasis, then cleaning the anogenital area after defecation from the anus towards the vagina (back to front) could introduce rectal trichomonads into the vagina and this would increase rates of trichomoniasis in those involved in this practice. This practice however was reported by only a few study participants and no such association was observed.

In this population of women studied, past treatment for a vaginal discharge even though significantly associated with infection, was not an independent predictor of infection when adjusted for other factors predictive of infection.

The diagnostic values of all factors predicting TV infection was poor. Even the 3 strongest predictors of infection, age less than 20 years, no religion and being of an

ethnic group other than the predominant groups in the study area, had poor sensitivities (3.7%-18.0%), and positive predictive values (12.3%-18.4%). Cotch and colleagues studying over 13000 pregnant women in the US have found similarly that demographic and behavioural factors predictive of TV infection were poor at selecting those who would be at high risk for infection. In their study, the sensitivities of the predictive factors smoking, black race and single marital status for TV infection were 42%, 68% and 75% respectively. However the positive predictive values for infection were 17.6%, 2.4% and 16.7% respectively [76].

All the variables independently predictive of infection were used in the definition of scores to determine the diagnostic value of a combination of such scores. Two scoring systems were investigated, one giving a unitary score for the presence of factors predicting infection, and the other, a weighted score depending on the odds ratio for infection of the factors. In general, both systems had poor predictive values for TV infection in the study population.

Even though the sensitivity of a score of 1 or more combinations for trichomoniasis was 97.7% using both scoring systems, the proportion of women who had this score(s) and who had trichomoniasis was only 5.4%. However, as many as 88.9% (3386 out of 3807) of women would have been selected for screening/treatment. This would not be cost effective. There would be significant over-treatment of the uninfected with cost implications, and worry over possible unwarranted adverse drug effects and antimicrobial resistance development. Similar sentiments have been expressed with the use of the syndromic approach for the management of cervicitis in populations with a low prevalence of sexually transmitted cervical infections [314].

Having high scores (6 in the simplified system, and 9 or more in weighted system) produced excellent predictive values. However in this population, a maximum of 2

out of the 3807 women would have been selected for testing and/or treatment, thus leaving out many infected women who would need treatment

In terms of good predictability for the selection of women most likely to be at risk for TV infection, there is not much to choose between the 2 scoring systems. Though the simplified system, needing less calculations would be easier to apply in a field setting, its outcome for the selection of infected women is minimal.

Women who were symptomatic for TV infection had significantly higher scores using both scoring systems than those asymptomatic. This unfortunately does not help the selection of those infected as a large proportion (38%) of the infected women were asymptomatic.

Although the variables identified as predictive of TV infection present a general profile of the women most likely to be infected with TV in this study, in such a low TV prevalent population, the utility of these variables as a basis for the selection of women for screening for TV or treatment is minimal.

One reason for the poor positive predictive values with these variables for trichomonas infection could be that individual factors such as complaint of vaginal discharge, young age, and douching have also been recognised with other reproductive tract infections in women, and alone would be poor predictors of TV infection. As these other infections were not controlled for in this study, it is difficult to judge their relative contributions.

Studying the relationship between socio-demographic, sexual behaviour and physical examination variables and TV infection among family planning clients in Kenya, Dally *et al* identified factors including unmarried status, more than 1 sexual partner in the past year, and vaginal discharge to be predictors of infection. Combinations of

these factors however, were poor at predicting infection and hence the selection of clients for testing for TV [315].

Only 2.3% (4/173) of the women testing positive for trichomoniasis did not fit any of the factors used in calculating the scores. This confirms the usefulness of the factors in ruling in infection.

Even though highly desirable, the unavailability of facilities and personnel and the cost of microbiological screening of vaginal trichomoniasis and other sexually transmitted infections in many developing countries including Ghana, currently precludes the use of the laboratory for the identification of STD related pathogens. In low STD prevalent settings, also with low prevalence of risk factors associated with vaginal/cervical infections, the development of treatment algorithms which could predict infection based on these risk factors for their empirical and/or syndromic management is next to impossible. A recent study from Ghana reported the prevalence of gonorrhoea and chlamydia infection at 1.5% and 0% respectively in non high risk women in the general population [316]. The present study has also highlighted the difficulty in identifying epidemiological factors that could predict TV infection. In such a population, the rational approach to management of vaginal discharge would be the identification of those infected through microbiological screening. With present laboratory tools for the diagnosis of TV and other STDs being relatively technologically demanding and not being affordable or with poor test performances, the need for cheaper alternatives is paramount.

4.11 Limitations to the Study

Inter-observer variability may bias any study if different study subjects are seen by different interviewers. The study tried to avoid or reduce this variability by pre-study

training of interviewers, "quality controlling" their work as detailed in Section 3.5, and also blinding them to the case/control status of the study participants. Nonetheless subjectivity in interpreting some of the responses of study subjects can not be ruled out.

Responses to aspects of the questionnaire examining sexual practices of the women (oral and anal sex) and their partners' fidelity, especially in face to face interviews as used in this study may not have been correctly given. Not only is it commonplace in many African societies for women to want to profess to society norms (that their partners are not seeing other women), they also believe it is an indictment on them personally and their marriage if their partners are seeing other women and so would not admit to it.

It is not unusual for women not to know whether or not their spouses have been treated for STIs in the past. Probably on the contrary, many men would not divulge this to their spouses. A difficulty for women in answering this could bias their responses.

The study could also suffer from recall bias as in all questionnaire based studies where responses rely on recalling past events.

5.0 RESULTS: CLINICAL CORRELATES OF VAGINAL TRICHOMONIASIS IN PREGNANT WOMEN

Clinical variables elucidated on genital examination were studied for their associations with *T.vaginalis* infection. The parameters examined were; presence of abnormal vaginal discharge, yeast-like discharge, offensive vaginal odour, the amount of discharge, colour of discharge and pH of the discharge. Vulva erythema, colpitis macularis (strawberry cervix), and faecal staining of the perineum were looked for.

Circumcision status of the women was checked and the amine test performed on vaginal discharges. As in the previous study all analyses were age adjusted for similar reasons.

A Gram stain also was done from one of the vagina swabs and Nugent's scoring criteria for the diagnosis of Bacterial vaginosis [304] determined. Using characteristics of the discharge, and clue cell results from above, the diagnosis of Bacterial vaginosis by Amsel's criteria [176] was also carried out.

5.1 Univariate and age-adjusted analysis of associations between Clinical parameters and *Trichomonas vaginalis* infection (Table 5.1)

Forty-three out of the 173 cases (24.9%) had an abnormal vaginal discharge, compared with the 13.1% of controls (p=0.001). A yeast-like discharge was found in 17.9% cases and in 9.8% controls (p=0.01). An offensive vaginal odour was present in 12.1% cases and 8.7% of controls (p=0.26).

The amount of vaginal discharge observed was categorised as normal (no obvious discharge seen), discharge seen within the vagina only and discharge seen at the

introitus/externally. In 76.3% of cases and 87.6% controls, no obvious discharge was seen. Discharge was seen within the vagina of 21.4% cases and 9% controls. Externally observed discharge (or at introitus) was reported from (2.3%) cases and (3.5%) controls. The amout of vaginal discharge was significantly associated with infection (p=0.004).

While 78.6% of cases had a normal coloured discharge (absent or colourless), this was the case for 89.6% of controls. A whitish, greyish, yellowish or greenish discharge was seen in 20.2% cases and 10.2% controls. The discharge was blood stained in 2 cases and 1 control respectively. An abnormally coloured (non-clear) discharge was significantly associated with infection (OR 2.19, 95%CI 1.3-3.5, p=0.001).

A reddened vulva was observed in 3 (1.7%) cases and none of controls, whilst the pathognomonic sign of strawberry cervix was seen in 6 (3.5%) of cases and no controls.

There was no faecal staining of the perineum of any case. This was seen in only 1 woman in the control group.

73.4% of cases and 82.4% of controls had a vaginal pH up to 5. Vaginal pH>5 was found in 26.6% of cases and 17.6% of controls. This (pH>5) was significantly associated with infection (OR 1.72, 95% CI 1.1-2.6, p=0.01).

Circumcision was not significantly associated with infection (OR=0.8, 95%CI 0.3-2.7, p=0.8). Of the circumcised, type 1 circumcision (only tip of clitoris cut) was observed in 4 cases (100%) and 11 controls (91.67%) whilst type 3 (excision of clitoris and part of labia) was observed in 1 control alone (8.3%).

A positive amine test was observed in 30% of cases and 22% of controls. This was associated with infection at borderline significance (OR 1.43, 95%CI 0.95-2.1, p=0.08)

By Amsel's criteria, 85.6% cases against 91.7% controls had bacterial vaginosis, whilst in using Nugent's criteria 83.8% cases as against 84.7% controls had bacterial vaginosis. Whilst the diagnosis of bacterial vaginosis by Amsel's criteria was significantly associated with TV infection (OR=1.78, 95%CI 1.0-3.2, p=0.04), diagnosis by Nugent's scoring system was not significantly associated with infection (OR=0.99 CI 0.6-1.6, p=0.9).

Table 5.1. Crude and age-adjusted analysis of association between TV infection and various clinical criteria on genital examination

Variable	**Cases n (%)**	**Controls n (%)**	**Crude OR**	**95% CI**	**Age adjusted OR**	**95% CI**
Abnormal vaginal discharge						
No	130 (75.1)	400 (86.9)	1		1	
Yes	43 (24.9)	60 (13.1)	2.20	1.4-3.4	2.10	1.3-3.2
Yeast-like discharge						
No	142 (82.1)	415 (90.2)	1		1	
Yes	31 (17.9)	45 (9.8)	2.01	1.2-3.3	1.92	1.1-3.1
Offensive vaginal discharge						
No	152 (87.9)	420 (91.3)	1		1	
Yes	21 (12.1)	40 (8.7)	1.45	0.8-2.5	1.39	0.7-2.4
Amount of discharge						
Normal	130 (76.3)	400 (87.6)	1		1	
Within vagina	37 (21.4)	41 (9)	2.75	1.7-4.5	2.66	1.6-4.3
At introitus	4 (2.3)	16 (3.5)	0.76	0.25-2.3	0.66	0.2-2.1

Colour of discharge						
Normal (clear)	136 (78.6)	412 (89.6)	1		1	
White/grey/ Yellow/green	37 (20.2)	48 (10.2)	2.33	1.4-3.7	2.19	1.3-3.5
pH > 5						
No	127 (73.4)	379 (82.4)	1	1.1-2.5	1	
Yes	46 (26.6)	81 (17.6)	1.69		1.72	1.1-2.6
Circumcision						
No	169 (97.7)	448 (97.4)	1		1	
Yes	4 (2.3)	12 (2.6)	0.80	0.3-2.7	0.80	0.3-2.7
Amine test						
Negative	121 (70)	359 (78)	1		1	
Positive	52 (30)	101 (22)	1.52	1.0-2.2	1.43	0.95-2.1
Bacterial vaginosis (Amsel)						
No	25 (14.4)	38 (8.3)	1	1		
Yes	148 (85.6)	422 (91.7)	1.88	1.0-3.2	1.78	1.0-3.1
Bacterial vaginosis (Nugent)						
No	28 (16.1)	70 (15.2)	1		1	
Yes	145 (83.8)	390 (84.7)	0.92	0.57-1.50	0.99	0.6-1.6

5.2 Multivariate analysis

Due to co-linearity between abnormal vaginal discharge and variables such as yeast-like discharge, increased quantity of discharge, white/grey/yellowish/greenish discharge, only abnormal vaginal discharge, discharge pH>5, and amine test were entered into the multivariate model.

On multivariate analysis, only abnormal vaginal discharge (OR=1.8, p=0.005) and pH > 5 (OR=1.5, p=0.05) remained independently associated with infection. Table 5.2 shows the final multiple logistic regression model.

Table 5.2. Final multiple logistic regression analysis of Clinical factors associated with TV infection

Variable	Adjusted OR	95% CI	P
Abnormal vaginal discharge			0.005
No	1		
Yes	1.87		
pH >5			0.05
No	1		
Yes	1.50		
Amine test			0.7
Negative	1		
Positive	1.09	0.7-1.7	

5.3 Diagnostic value of factors predictive of infection

The sensitivity, specificity and predictive values of the factors independently predicting TV infection were calculated for the target population. As shown in Table 24, individually, each of the factors predictive of TV infection had a poor positive predictive value for infection in the study population (Table 5.3).

Table 5.3. Diagnostic value of clinical factors independently predictive of TV infection

Factor	Sensitivity (%)	Specificity (%)	Positive predictive value in target population (%)	Negative predictive value in target population (%)
Abnormal vaginal discharge	25.0	86.9	9.0	95.7
pH >5	26.6	82.4	7.3	95.6

5.4 Binary scoring

Sores of 1 and 0 were assigned to each of the women examined in respect of whether or not there was the presence of any of the clinical parameters independently

associated with TV infection, with 1 for the presence of the factor and 0 if it was absent. Total score per subject was calculated. A score of 1 for a subject meant they had only one out of the 2 factors independently associated with infection etc (Table 5.4). A maximum score of 2 is the maximum score attainable.

Distribution of scores among cases and control cohort is shown in Table 5.4.

Table 5.4. Score distribution among cases and controls.

Score	**Cases (%)**	**Controls (%)**	**Total**
0	104 (60.1)	337 73.2)	441
1	49 (28.3)	105 (22.8)	154
2	20 (11.6)	18 (3.9)	38
Total	173 (100)	460 (100)	633

About 60% (104/173) of women with trichomoniasis did not have any of the parameters independently predicting infection. While 39.9% of cases (69/173) had one or both of the 2 factors associated with infection, this was the case for 26.7% of controls.

5.5 Diagnostic value of scoring system

Cut-off score points from the combination of scores, was used to calculate the sensitivity, specificity and positive predictive value for the selection of patients with *T. vaginalis* infection (Table 5.5). Calculation of the diagnostic values was adjusted for all women in the study population. The number of women selected for each cut-off point is also shown.

Table 5.5. Diagnostic value of cut off combination scores of Clinical factors

Cut-off score	**Sensitivity (%)**	**Specificity (%)**	**Positive predictive value in target population (%)**	**Negative predictive value in target population (%)**	**Number of women selected in study population n=3807 (%)**
> = 1	39.9	73.2	7.1	95.9	1043 (27.4)
2	11.7	96.0	13.4	95.4	164 (4.3)

The clinical utility of these scores is limited for the selection of patients with trichomoniasis. For subjects with scores, the highest sensitivity was 39.9% and the highest positive predictive value of a score was 13.4%.Though the proportion of subjects with various combined scores who would have *Trichomonas vaginalis* infection (positive predictive value) increased with increasing combined scores, the percentage of women in the population who would have been selected for treatment or screening based on these scores decreased with increasing score. The combination with the greatest sensitivity if a score was recorded (39.9%) suggests that women who have at least 1 of the 2 variables scored should be selected; this subgroup represented 27.4% of the study population.

The use of the parameters in ruling in disease was good for scores of 2 (96%) but poor for the presence of only 1 factor (73%).

Table 5.6. Score distribution among women in the case-control cohort who were symptomatic and asymptomatic for TV infection

Score	**Symptomatic n(%)**	**Asymptomatic n(%)**	**Total**
0	205 (46.9)	236 (53.5)	441 (100)
1	98 (63.6)	56(36.4)	154 (100)
2	35 (92.1)	3 (7.9)	38 (100)
Total	338	295	633

Significantly more symptomatic women had scores (1 or 2) compared to asymptomatics ($p<0.0001$). The odds ratio of being symptomatic with a score was 2.48 (95% CI 1.8-3.3, $p<0.0001$).

5.6 DISCUSSION: CLINICAL CORRELATES OF VAGINAL TRICHOMONIASIS IN PREGNANT WOMEN

As a sequel to the identification of epidemiological factors for the prediction of TV infection, this part of the study was undertaken to identify clinical parameters that could also predict infection with the same aim as previously i.e for the selection of subjects for screening and /or treatment of infection.

There have been conflicting reports of the association between trichomoniasis and signs of abnormal vaginal discharge. Studying high risk populations, Spence *et al* [224] and Fouts and Kraus [101] did not find this association. However, McLelan *et al* [91] and Wolner-Hansen *et al* [317] reported finding this association in similar populations attending STI clinics. Importantly in all these studies has been the different definition for abnormal vaginal discharge used. This has meant various combinations of increased quantity, frothiness, colour, offensive odour and itch. Different combinations used in different studies could alter associations. In this present study, a more liberal definition encompassing one or more of the above signs was used. A significant association was detected between infection and abnormal discharge. On testing associations between these individual constituents of abnormal discharge and TV infection, all but offensive vaginal odour remained significantly associated with infection. An offensive vaginal odour, though not significantly associated with TV in this study (p=0.2), has been significantly associated with TV in other studies[317].

In this study, a yeast-like discharge was documented more frequently in women with trichomoniasis (OR=2). The finding of co-infections with multiple reproductive tract pathogens is not unusual, and especially in this population of pregnant women, could be expected, as yeast infections are quite common in pregnancy due to changing

hormonal levels. In non-pregnant women, yeasts have been detected less frequently in those with trichomoniasis, a possible mechanism being an alkaline environment created by the trichomonads, which ecology is not favoured by yeasts which thrive in an acidic environment [318].

Colpitis macularis or strawberry cervix was identified in 6 (3.5%) of cases and none of the controls. Lang and Ludmir in 1961 described this sign as pathognomonic for trichomoniasis having found it in 78% of women with trichomoniasis and none of controls [319]. Increased yield of this sign is observed when a colposcope is used [319, 320]. This study's findings are in conformity with Fouts and Kraus and Wolner-Hanssen who reported a 2.3% and 1.7% rate among cases with the unaided eye [101, 317].

Though the finding of strawberry cervix was highly significant for TV infection, ($p<0.001$, Fishers exact test) its clinical utility for the prediction of infection was low. This sign had a 73.37% sensitivity (95%CI=69.7-76.8) and 100% specificity (95%CI=54-100), and a positive predictive value of only 3.5% in this low TV prevalent population. Using this sign alone, only 0.95% of the study subjects would have been selected. In Wolner-Hanssen's study of the population attending a sexually transmitted diseases clinic in the US with 15% TV prevalence, the sensitivity and specificity of colpitis macularis was reported as 44% [317] and 99% respectively. Its positive predictive value for TV infection was 90%. The high positive predictive value in this population compared to that of the pregnant women in Kumasi could be due most likely to the symptomatic status of the selected patients attending the STI clinic.

The association between TV infection and ano-genital hygiene was further explored, by examining faecal staining of the perineal region in the women. The prevalence of TV has been related to poor sanitary facilities [321] and it has also been suggested that

poor ano-genital hygiene could be a reason for the differential racial prevalence of TV infection [100]. Unfortunately, this study population could not be used to study this assertion as the perineum of almost all study participants was "clean" on examination. It is a common practice in Ghana for women attending health facilities where there is the possibility of genital examination not only to wash and clean the genital area before attending but also to use new underwear. Also health education talks given by midwives at antenatal clinics stress to the women the importance of general and genital hygiene.

This study with others confirms the association between TV infection and a shift of vaginal pH towards alkalinity (pH>5).

Together with a pH>5, a homogenous vaginal discharge, a positive amine test and the finding of clue cells on microscopy of vaginal discharge are components used in the diagnosis of bacterial vaginosis (BV) by Amsel's criteria [176]. The presence of 3 out of these 4 criteria constitutes a diagnosis. Another diagnostic criterion, Nugent's scoring system [304] is based on morphological types of organisms found in the vagina. A shift of these morphotypes from the normal Gram positive lactobacilli predominant flora to one of Gram variable rods (*Gardnerella sp*) and Gram negative curved rods (*Mobiluncus sp*) informs the diagnosis of BV. Most often these abnormal morphotypes are found cluttered on and around the edges of vaginal epithelial cells, thus obscuring the fine edges. Referred to then as "clue cells", some authors deem their presence alone indicative of BV [322].

This study demonstrated the association between TV and BV as defined by Amsel[176]. Similar associations have been reported previously [317, 323] . However as defined by Nugent, the association was not observed. In comparing 5 different criteria for the diagnosis of BV (including Amsel and Nugent systems) and assessing their

association with TV infection in an antenatal setting, James *et al* reported that TV was not frequently associated with BV, saying the women had some of the characteristics that met the criteria for BV such as profuse discharge and elevated vaginal pH (Amsel), but that they usually had no clue cells [324]. Clue cells identification could be the reason behind discordancy between the associations of TV and the 2 BV diagnostic systems in the Kumasi study. A pH>5, and a positive amine test, both components of Amsel's criteria were significantly associated with TV infection in the Kumasi study. Homogenous vaginal discharge, the third criterion could pass for increased amount of discharge and/or a non-clear discharge both of which were significantly associated with TV infection. With these 3 criteria associated with TV infection, a diagnosis of BV according to Amsel can be made. The identification of clue cells and scoring on Nugent's criteria can be technically demanding, requiring an experienced microscopist. Any misreading of smears would have an impact on BV diagnosis by this criterion, as correctly identifying and scoring the morphotypes is the sole criterion for diagnosis. In the study laboratory in Kumasi, Ghana, this was the first time people had been trained to score BV by this system. Even though a quality control system was in place where 10% of slides were re-read and judged satisfactory, it is not unusual that mistakes and misreading could occur.

An exploration of the clinical utility of these signs independently associated with infection (abnormal vaginal discharge and pH >5) revealed poor predictability for the selection of TV infected patients. The relatively low positive predictive value of the signs (9.0% and 7.3%, respectively) could partly be explained by the frequent occurrence of these signs among women who may have other reproductive tract infections apart from trichomoniasis. For example abnormal vaginal discharge in the

study women was not only associated with trichomoniasis, but was also highly associated with a yeast-like discharge (OR=7.2, 95% CI=3.8-13.7, p<0.001). Also in this study population, factors such a pH>5, positive amine test and increased amount of discharge all of which were significantly associated with TV, could collectively pass for a diagnosis of Bacterial vaginosis [176]. Simultaneous occurrence of more than one reproductive tract disease in an individual is not uncommon [101].

The scores derived in this study selects a maximum of 39.9% of those infected for the objectives of this study. This is far lower than the least sensitive laboratory test currently used for detecting TV infections. For women in this study, the wet prep technique had a sensitivity of 81.5% (141 of 173). The target population positive predictive value is also poor at selecting TV infected women.

Several studies among pregnant women in developing countries have evaluated the use of scoring systems for the diagnosis of cervical infection [325, 326, 327]. Some of these studies have included the use of clinical signs. Unfortunately as seen in the present study, the performance of these algorithms has not been encouraging.

In conclusion, even though certain clinical parameters i.e. an abnormal vaginal discharge and pH>5 have been found to be independently associated with TV infection in this group of pregnant women, their utility for the prediction of infection in order to allow for the selection of potentially infected women in this population for screening and/or treatment of infection is limited. The search for simple, reliable, rapid and developing country-friendly diagnostics for detection of infection remains a priority for the control of vaginitis due to *T.vaginalis*.

5.7 Limitations to this study

Two female examiners, who were blinded to the case-control status of the women, were trained and examined the women reporting on a uniform pro-forma. Inter-observer variability can not be ruled out. There could also have been some subjectivity in the definitions given to some of the parameters studied e.g. vaginal odour and amount of discharge. These did not depend on any objective criteria, and for example there could be a fine line between a normal vaginal discharge (no discharge seen) and a scanty vaginal discharge (discharge in the vagina) which could be reported differently.

6.0 RESULTS: COMBINED SCORES USING SOCIO-DEMOGRAPHIC, BEHAVIOURAL AND CLINICAL PARAMETERS PREDICTING TV INFECTION IN PREGNANT WOMEN

In trying to improve the predictive value for TV infection, a combination of scores of factors predicting infection (socio-demographic, behavioural and clinical) was investigated. The weighted scoring system for the socio-demo variables was used. A maximum score of 16 is attainable.

Table 6.1 shows distribution of cases and controls in the combined scores.

Table 6.1. Score distribution among cases and controls combining socio-demographic and clinical factors predicting TV infection.

Score	Cases (%)	Controls (%)	Total (%)
0	4 (8.0)	46 (92.0)	50 (100)
1	21 (13.5)	135 (86.5)	156 (100)
2	39 (24.1)	123 (75.9)	162 (100)
3	35 (31.5)	76 (68.5)	111 (100)
4	25 (38.5)	40 (61.5)	65 (100)
5	24 (50.0)	24 (50.0)	48 (100)
6	13 (54.2)	11 (45.8)	24 (100)
7	8 (72.7)	3 (27.3)	11 (100)
8	1 (33.3)	2 (66.7)	3 (100)
9	2 (100)	0 (0)	2 (100)
14	1 (100)	0 (0)	1 (100)
Total	173 (27.3)	460 (72.7)	633 (100)

As seen with the previous systems, the lower end of the combined scores (0-4) was predominantly controls (77.2%), whilst the upper end (6-14) was made up mostly of cases (70%). Equal proportions of cases and controls scored midway (score 5)

6.1 Diagnostic value of combined scores

The sensitivity, specificity and target population predictive values of each cut-off of the combination of scores (Table 6.2) was determined as previously.

Table 6.2. Performance characteristics of combined cut-off scores

Cut-off score	**Sensitivity (%)**	**Specificity (%)**	**Positive predictive value in target population (%)**	**Negative predictive value in target population (%)**	**Number of women selected in study population n=3807 (%)**
> = 1	97.3	10.0	5.31	98.6	3440 (90.5)
>=2	85.6	39.3	6.8	97.8	2356 (61.8)
>=3	62.8	71.5	8.1	97.3	1149 (30.2)
>=4	42.5	82.6	11.3	96.5	706(18.6)
>=5	28.2	91.2	14.4	96.0	368 (9.7)
>=6	14.4	96.5	17.6	95.6	153 (4.0)
>=7	7.0	98.9	25.0	95.3	52 (1.4)
>=8	2.1	99.6	20	95.1	20 (0.5)
>=9	1.9	100	100	95.1	3 (0.08)
14	0.53	100	100	95.0	1 (0.03)

Whilst the sensitivity of the cut-off scores decreased with increasing combined scores, the positive predictive value increased likewise. The combinations achieving the highest PPV (>=9->=14) were poorly represented in the study population (1-3 women). In contrast, a large representation of women in the study population was observed in combinations of scores with low PPV.

6.2 Score distribution among women symptomatic and asymptomatic for TV infection

About 8% (50/633) of the women did not achieve any score with the combination. Within each score, asymptomatics were the majority among lower scores (0-1), while women with symptoms were the majority from score 2 or more (Table 6.3).

Table 6.3. Score distribution among case-control women symptomatic/asymptomatic for infection.

Score	Symptomatic n (%)	Asymptomatic n(%)	Total
0	1 (2.0)	49 (98.0)	50 (100)
1	52 (33.3)	104 (66.7)	156 (100)
2	85 (52.5)	77 (47.5)	162 (100)
3	87 (78.4)	24 (21.6)	111 (100)
4	45 (69.2)	20 (30.8)	65 (100)
5	32 (66.7)	16 (33.3)	48 (100)
6	20 (83.3)	4 (16.7)	24 (100)
7	11 (100)	0 (0)	11 (100)
8	2 (66.7)	1 (33.3)	3 (100)
9	2 (100)	0 (0)	2 (100)
14	1 (100)	0 (0)	1 (100)
Total	338 (100)	295 (100)	633 (100)

6.3 DISCUSSION: COMBINED SCORES USING SOCIO-DEMOGRAPHIC, BEHAVIOURAL AND CLINICAL PARAMETERS PREDICTING TV INFECTION IN PREGNANT WOMEN

As in the 2 preceding chapters, both socio-demographic and clinical parameters which independently predicted TV infection, performed poorly when combined and their diagnostic value for infection calculated.

With 90.5% of the study population having at least 1 of an attainable 16 scores, the majority of the women qualified to be selected for the purposes of being screened or treated for TV infection, a situation which would not give value for money. Reasons for this poor predictability of infection could be the low prevalence of TV in the population. It could also be due to the association of these factors with other reproductive tract infections in the women as discussed previously. Infection could be predicted in women who had up to 9 or the maximum 14 scores. These women however made up less than 1% of the population of women studied.

Thus again, the utility of selecting subjects for screening and/or treatment of TV infection based on a combination of demographic and clinical factors predicting TV infection was minimal. The use of these scoring systems for the screening and/or diagnosis of TV infection in this group of women is thus not worthwhile.

7.0 RESULTS: COMPARISON OF DIAGNOSTIC METHODS FOR THE DETECTION OF TV

Various diagnostic tests were compared for the detection of *Trichomonas vaginalis* infection. These included 2 traditional diagnostic tests (wet prep examination and culture) and 3 others ; latex agglutination (LAT), Enzyme immunoassay(EIA), and PCR. Towards the end of the study, Kalon Biological (Surrey, UK) sent in their newly developed lateral flow dipstick test also for evaluation.

An expanded gold standard (EGS) of culture and/or wet prep was used as the reference test.

7.1 Test results among selected women

Of the 3807 women in the target population who had LAT done on their vaginal swabs, 206 and 3601 were positive and negative respectively. However, only 633 samples (all 206 positives and 427 negatives) were selected for the case control study and these samples were subjected to testing by wet prep, culture, EIA, PCR and lateral flow techniques.

A 2X2 table of results of the EGS and LAT for the case-control group is shown in Table 7.1.

Table 7.1. Results of LAT and expanded gold standard in case-control population

LAT	EGS		Total
	Positive	Negative	
Positive	171	35	206
Negative	2	425	427
Total	173	460	633

7.2 Estimated gold standard results in target population

In estimating the predictive values of LAT in the target population, an estimate of the number of women in the target population who would have tested positive on the EGS was done. In doing this, it was assumed that women in the target population who were not selected for the case control study would not differ in terms of the gold standard tests from those selected.

Of the 427 women testing negative for LAT in the case-control cohort, 2 were positive by the EGS tests. Thus it was estimated that for the 3601 women in the target population testing negative for LAT, 2x (3601/427) =17 would be positive by the gold standard test. This implies that 3584 would be true negative (=3601- 17). Putting these estimated figures into a table, Table 7.2 is derived.

Table 7.2. Estimated results of LAT and the EGS in the target population

LAT	EGS		Total
	Positive	Negative	
Positive	171	35	206
Negative	17	3584	3601
Total	188	3619	3807

Thus, in the target population, an estimated 188 and 3619 women would be positive and negative respectively by the EGS. Hence, the estimated population prevalence of TV by the EGS would then be 188/3807 = 4.93% (95%CI 4.3-5.7).

The sensitivity, specificity, and predictive values of the diagnostic tests compared are derived for the study target population. Target population adjusted figures are therefore made from results of the case control cohort.

7.3 Sensitivity, specificity and predictive values of the Latex agglutination test (LAT)

From Table 7.1, of the 633 vaginal swab eluates, 206 (32.54%) were positive and 427 (67.46%) negative on latex testing. 83% (171) of the positives were study cases and 35 (17%) were controls. Whilst 98.9% (171) of cases were latex positive, 2 (1.1%) were latex negative.

Significantly more women who were symptomatic were latex positive (p=0.04)

Of the 35 samples which were false positive by LAT, all but 5 were negative by other TV diagnostic tests – EIA and PCR. All the 5 were positive by PCR, and 2 of the 5 were also positive by EIA. None of the 35 samples was also positive by EIA alone.

The sensitivity of LAT in the target population was 91.0% (95%CI 85.9-94.6%), and specificity 99.0% (95% CI 98.7% – 99.3%). The predictive value of a positive test was 83.0% (95% CI 77.1% - 87.9%), and that of a negative test was 99.5% (95%CI 99.2% - 99.7%).

Within tests agreement (Kappa statistic) of the LAT and the EGS was 0.93 (95%CI 0.91-0.95)

7.4 Sensitivity, specificity and predictive values of the Wet prep test

141 of the selected 633 swabs were positive and 492 negative on wet prep direct microscopy. All those testing positive were cases. By definition of the EGS, none of the controls were test positive (Table 7.3). Of the study cases, 141 (81.5 %) were wet prep positive and 32 (18.5%) wet prep negative. 63% of wet prep positive subjects were symptomatic and 37% asymptomatic (χ^2 =6.9, p=0.009).

Table 7.3. Comparison of the wet prep with the expanded gold standard in the case-control cohort

Wet prep	EGS		Total
	Positive	Negative	
Positive	141	0	141
Negative	32	460	492
Total	173	460	633

Target population test results are compared with the EGS in Table 7.4.

Table 7.4. Estimated results of wet prep and the EGS in the target population

Wet prep	EGS		Total
	Positive	Negative	
Positive	154	0	154
Negative	34	3619	3653
Total	188	3619	3807

Wet prep test performances were: sensitivity 77.8% (95%CI 71.3-83.4), specificity 100% (95%CI 99.99-100), positive predictive value 100% (95%CI 97.6-100), and negative predictive value 98.8 (95%CI 98.4%-99.1%)

The Kappa statistic for wet prep and the EGS was 0.94 (95%CI 0.93-0.95)

7.5 Sensitivity, specificity and predictive values of Culture

170 of the 633 swabs were positive on InPouch culture (Table 7.5). These made up 98.26% (170/173) of the study cases. 3 study cases were negative by culture, but wet prep positive. 2 of these 3 samples were also positive by latex agglutination, EIA and PCR. The third was positive by PCR, but negative on testing by the 2 immunologically based assays – LAT and EIA.

Of the women testing positive on culture, significantly more were symptomatics compared to asymptomatics (62% vrs 38%, p=0.01).

Table 7.5. Comparison of culture with the expanded gold standard in the case-control cohort

Culture	EGS		Total
	Positive	Negative	
Positive	170	0	170
Negative	3	460	463
Total	173	460	633

The target population performance of culture compared to the gold standard is shown in Table 7.6.

Table 7.6. Estimated results of culture and the EGS in the target population

Culture	EGS		Total
	Positive	Negative	
Positive	185	0	185
Negative	3	3619	3622
Total	188	3619	3807

The sensitivity of culture was 98.4% (95%CI 95.4-99.7) and specificity 100% (95%CI 99.9-100). Mirroring specificity was a positive predictive value of 100% (95%CI 97.8-100). The predictive value of a negative test was 99.9% (95%CI 99.7-99.9).

A kappa index of 0.98 (95%CI 0.97-0.988) was observed between culture and the EGS.

7.6 Sensitivity, specificity and predictive values of the EIA test

Due to loss from spills and other circumstances, only 560 test samples (153 cases, 407 controls) were available for EIA testing. Of these, 119 tested positive and 441 negative. 113 (95%) of the positives were samples from cases and 6 (5%) from

controls. Of the 153 cases, 40 (26 %) were negative by EIA testing and 74% (113) positive on testing. 6 (1.5%) women without infection were positive on testing.

EIA test performance is shown in Table 7.7.

Table 7.7. Comparison of EIA test with the expanded gold standard of study cohort

EIA	EGS		Total
	Positive	Negative	
Positive	113	6	119
Negative	40	401	441
Total	153	407	560

Adjusted figures for the target population are shown in Table 7.8.

Table 7.8. Estimated results of EIA and for the EGS in the target population

EIA	EGS		Tota!
	Positive	Negative	
Positive	139	53	192
Negative	49	3566	3615
Total	188	3619	3807

Sensitivity of EIA as a TV diagnostic in this population was 73.9 (95%CI 67.0-80.0) and specificity 98.5% (95%CI 98.0-98.9). The PPV was 72.4% (95%CI 65.5-78.6) and NPV 98.6% (95% CI 98.2– 99.0)

The Kappa index between EIA and the EGS was 0.9 (95%CI 0.89-0.91).

7.7 Sensitivity, specificity and predictive values of the PCR

The quality of vaginal swabs for PCR assays was determined by the presence of β-2 microglobulin gene in each swab sample. β-2 microglobulin was present in 600 of the 633 swabs when assayed neat. 33 swabs were negative for this assay. When a 1:10 dilution of these 33 swab samples were assayed, this had diluted out the PCR

inhibitors and β-2 presence was confirmed in 21. The rest (12) were deemed either as having inhibitors of PCR assay or not having adequate samples for analysis. Thus 621 samples were available for PCR analysis for the presence of *T.vaginalis*. The 621 samples comprised 161 cases and 460 controls (Table7.9).

There was a 100% agreement between results of the 2 PCR assays for *T.vaginalis* ie using Kengne and Shaio primers respectively. All 191 samples positive on screening with the Kengne primers were confirmed as positive with the Shaio primers. Similar results were obtained with samples testing negative (430).

Table 7.9. Comparison of results of PCR assay with the expanded gold standard in the case-control group

PCR	EGS		Total
	Positive	Negative	
Positive	142	49	191
Negative	19	411	430
Total	161	460	621

142 (88.2%) of cases were PCR positive and 19 (11.8%) were PCR negative. 49 uninfected women by the gold standard test were PCR positive.

Respective figures for the target population with TV prevalence at 4.93% are (Table 7.10):

Table 7.10. Estimated results of PCR and the EGS in the target population

PCR	EGS		Total
	Positive	Negative	
Positive	165	384	549
Negative	22	3224	3246
Total	187	3608	3795

Whilst the sensitivity of the PCR assay was 88.2% (95%CI 82.7- 92.4), the specificity was 89.4% (95%CI 88.3-90.3). The positive and negative predictive values for infection were 30% (95%CI 26.3-34) and 99.3% (95%CI 98.9-99.6) respectively.

The Kappa index of test agreement between PCR and the EGS was 0.89 (95%CI 0.88-0.90)

7.8 Lateral flow (Dipstick)

Evaluation of the performance of the Kalon lateral flow kit was incomplete. This was due to poor results obtained early on in the evaluation.

150 test samples made up of 75 cases and 75 controls were selected for the evaluation. Analysis of the results after 100 samples (70 cases and 30 controls) had been evaluated showed all negative samples (controls) tested negative. However, 48 of the 70 case samples (68.6%) tested negative. A decision to terminate further evaluation was taken in order to conserve vaginal swab eluates which were running low.

7.9 Summary of comparison of test performances of the TV Diagnostic tests.

Table 7.11 is a composite table showing the performances of all the diagnostic tests

Table 7.11. Summary of performance characteristics of diagnostic tests for *T.vaginalis*.

Diagnostic test	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**Positive predictive value % (95% CI)**	**Negative predictive value % (95% CI)**
Latex agglutination	91.0 (85.9-94.6)	99.0 (98.7-99.3)	83.0 (77.2-87.9)	99.5 (99.2-99.7)
Wet prep	77.8 (71.3-83.4)	100 (99.2-100)	100 (97.4-100)	98.8 (98.4-99.1)
Culture	98.4 (95.4-99.7)	100 (99.9-100)	100 (97.8-100)	99.9 (99.7-99.9)
Enzyme Immunoasay	73.9 (67.0-80.0)	98.5 (98.0-98.9)	72.4 (65.5-78.6)	98.6 (98.2-99.0)
Polymerase chain reaction	88.2 (82.7-92.4)	89.4 (88.3-90.3)	30 (26.3-34)	99.3 (98.9-99.6)

7.9 DISCUSSION: COMPARISON OF DIAGNOSTIC METHODS FOR THE DETECTION OF TV

Results from the previous chapters have highlighted the importance of laboratory support in the diagnosis of TV infection to accurately identify infected individuals. In a low TV prevalent population as that studied, this could be important in overcoming problems of over-treatment and under diagnosis.

The reference test used in this study was a combination of wet mount microscopy and/or culture. Even though culture of TV is the recognised gold standard in TV diagnosis, in situations where transportation of samples for culture over a long period has to be done before incubation, the delay could render trophozoites previously viable, unviable. To circumvent this, an experienced microscopist read wet mount preparations of swabs shortly after collection, and these results together with results of cultures carried out within 1 hour of sample collection informed the expanded gold standard.

All the tests evaluated had good specificities ranging from 90-100%. This is as reported in the literature [196]. By definition of the reference standard, the specificities of wet prep and culture were excellent.

The 98.4% sensitivity of culture in reality compares it to wet prep results in the reference standard. This result is in consonance with others published on the InPouch [212, 328] and confirms the superiority of culture against other diagnostics for TV. However, TV culture is not in generalised use worldwide due to reasons discussed earlier.

The 77.8% sensitivity of wet prep is also in conformity with other published studies [101, 196, 197] confirming its relative poor performance at detecting TV. Despite this, the

technique continues to be widely used in TV diagnosis because it can be done in a short time to allow same day treatment. Its performance however is highly dependent on the skills of the technician.

Three samples with flagellates having the characteristic morphology and motility of TV did not grow in culture. A probable reason could be loss of viability with delays in incubation. This was confirmed by the detection of TV by PCR genomic analysis in these 3 samples. This finding upholds the use of the expanded gold standard of wet prep and/or culture as reference the test.

EIA of vaginal swab eluates is one of the immunological techniques used in TV diagnosis. Its most appealing feature is the batch processing of samples which could be useful in field research situations. Even though the assay takes only about an hour to perform, as a day to day TV diagnostic tool its use may not be feasible due to the batch processing. The 74% sensitivity found in this study follows closely the reported pooled sensitivity of 82% (95%CI 74-90) in a recent review of diagnostic tools for TV [198]. Processing samples for EIA could be technically demanding, requiring trained technicians. In resource poor settings such personnel may not be available.

The latex agglutination test (LAT) performed creditably in this diagnostic comparison of TV tests. With a sensitivity of 91%, it compares favourably with culture. Carney *et al* reported 95% sensitivity (gold standard - wet prep and/or culture) using the LAT in a STI based population in the UK [22], which population would presumably be more symptomatic of infection than the pregnant women in the Kumasi study. Again, whilst vaginal swabs in the UK study were physician obtained via specula, those in Kumasi were self obtained by the women. The 35 women who had false positive results by this test dropped the positive predictive value of LAT to

83%. However, 5 of these women tested positive by the nucleic acid based PCR assay. Taking account of this would raise the positive predictive value marginally to 86%.

Compared to the commonly used wet prep examination, the LAT detected more women infected with TV (13% more). Its detection rate fell 7.4% behind culture. Various attributes of the LAT should make its use more widespread than the wet prep, especially in resource poor settings. The test is simple to perform, requiring no equipment other than a glass slide and mixing sticks, all of which are included in the kit. Results are obtainable within 3 minutes of sample and reagent addition, which should enable early and same day treatment of cases, a measure important in STI control. Skills to perform the test are minimal. Secondary school graduates have been trained to perform and read the test with little supervision. At a present cost of £1 per test, it is cheaper than other rapid tests for reproductive health use e.g. rapid *Chlamydia trachomatis* point of care tests. With bulk purchasing and use, it could even be cheaper. One other important issue is that the vaginal swabs were self obtained by the women for the LAT, removing issues of "invasive procedures". This should appeal in clinical settings where female genital examination is impossible or not needed.

These attributes again make the use of the LAT more favourable than culture, which apart from requiring trained and skilled technicians does not allow same day treatment, thus allowing infected asymptomatic people to transmit infection whilst awaiting results. The cost of return for culture results could also be a disincentive for people awaiting results. Thus even though culture detects 7.4% more infected people, its use could result in few patients being treated because they may not turn up for their results. Indeed work by Mukenge *et al* and Gift *et al* have demonstrated that a

low sensitivity point of care diagnostic tool can result in more infected people being treated than laboratory tests which involve a return visit to the health facility [329, 330.] This has often been termed the rapid test paradox.

In its quest for rapid diagnostic tests for use in STI settings in resource limited areas, the Sexually Transmitted Diseases Diagnostic Initiative (SDI) of the WHO lists 7 main qualities of such tests [331]; affordable, sensitive, specific, user friendly, rapid, robust and easy delivery to the setting. The LAT meets many of these criteria. Its sensitivity, specificity, user friendliness, and rapidity in performance have been discussed. Its present cost of £1, which could be lowered further, is not far from being affordable. Presently the unused kits are stored at 4°C. However they have been used under field temperatures of up to 28°C for 3-4 hours with no reports of underperformance, though the long term effects are unknown.

The good performance characteristics of the LAT in a resource limited setting make it an ideal choice in screening and/or diagnostic programmes for TV in the developing world. With demographic and clinical parameters being poor predictors of infection, such a laboratory diagnostic tool could be helpful in TV control programmes.

The PCR assay performed poorly compared to culture and the reference standard. Some reasons can be adduced. The reference standard definition inherently biased and made false positive all samples which were PCR positive but reference standard negative. The difficulty in evaluating a technique such as PCR using a traditional reference standard to which PCR may be more sensitive is recognised. Such were the difficulties in the early days when molecular diagnostic tests for *Chlamydia trachomatis* were being evaluated. 49 such samples were positive on analysis by 2 different PCR assays with primers targeting different parts of the genome of TV. In

some studies comparing PCR to the traditional tests, wet prep and culture using clinical samples, the gold standard has been positivity with the traditional tests and/or 2 PCR assays (with 2 different sets of primers) [256, 257]. The sensitivity and specificity of PCR in this study could have been greatly improved if such definition was incorporated.

19 samples positive by the traditional tests were negative on PCR. In comparing 5 different PCR assays for the detection of TV, Crucitti *et al* [257] showed that samples did not amplify uniformly with all primer sets. While some samples amplified with certain primer sets, others did not. Genetic variability could be responsible. In the Kumasi study, samples which amplified with Kengne primers, also amplified with Shaio primers. It is however not inconceivable that some samples in which there were no amplification with either primer sets are strains in which the target sequences were absent. One other possibility is inadequate sampling of the swabs for PCR analysis. Even though the human β-2 gene from cellular material could be amplified in these samples, inadequate sampling could arise from no TV on swab stick. Finally, the possibility of laboratory errors leading to misdiagnosis cannot be discounted. The 88.2% sensitivity of PCR in this study however, is generally consistent with other studies [250, 251, 252, 256, 332].

It was unfortunate the decision to terminate evaluation of the lateral flow kit. This was basically due to declining test sample volumes in the presence of an apparently poor performing kit. One reason for poor performance could have been degradation of protein antigens in the positive samples due to repeated freeze-thaw procedures. After immediate performance of the LAT on sample collection in Kumasi, samples stayed in the tropical sun for about 30 minutes – 1 hour before being stored at -20 °C. They were then transported on ice to London, where there were several freeze–thaw

procedures for the performance of EIA and the lateral flow testing. Proteins are known to degrade during such procedures and this could have affected test performance. The poor performance could also be due to poor sensitivity of the dipstick in detecting TV from swab eluates arising from low concentration of monoclonal antibodies for detection of the TV antigens.

Another antigen-based rapid point of care lateral flow kit, similar to that evaluated above, has been approved by the Food and Drugs Administration (FDA) of the US with purported sensitivity and specificity of 100% [333]. There has been no reported evaluation outside the US. Currently a test costs in the order of $10. Its performance in the developing world could further inform choice and expand the gamut of TV rapid tests available for use worldwide.

8.0 RESULTS: ARE TRICHOMONADS OTHER THAN TV INVOLVED IN THE AETIOLOGY OF VAGINAL TRICHOMONIASIS?

In this investigation, all samples for which TV was diagnosed by traditional tests i.e wet mount and/or culture were also subjected to testing for *P.hominis*, *T.tenax* and *T.vaginalis* by specific PCR assays.

All the 3 species specific PCRs for each sample were done from eluates of a single vaginal swab collected at the same time as swabs collected for wet prep and culture.

Of the 173 samples in which TV was detected by wet prep and/or culture, only 161 could be evaluated by PCR. This is because the human β-2 gene, the presence of which attests to sample collection adequacy and/or absence of PCR assay inhibitors, could not be amplified even after dilution in 12 samples. No PCR analysis with any of the trichomonad primers was carried out on these 12 samples.

In 152 of the 161 samples, PCR analysis for TV was positive. *P.hominis* and *T.tenax* were not detected by PCR in any of the samples, including the 9 samples for which PCR for *T.vaginalis* was negative, though wet prep and/or culture detected *T.vaginalis* by morphological appearance.

Table 8.1. Comparison of TV detection by traditional methods and specific trichomonad PCR assays of vaginal swab eluates.

	TV PCR Positive Negative	Ph PCR positive	Tt PCR positive
TV detected by Wet prep/culture	152 9	0	0

In summary (Table 8.1), 87.9% of samples positive for TV by wet prep and/or culture were also positive by TV PCR. No sample positive for TV by wet prep/culture was positive for *P.hominis* or *T.tenax* by PCR.

8.1 DISCUSSION: ARE TRICHOMONADS OTHER THAN TV INVOLVED IN THE AETIOLOGY OF VAGINAL TRICHOMONIASIS?

There have been speculations since the 1940s of the probable involvement of other human trichomonads (especially rectal trichomonads) in the aetiology of vaginal trichomoniasis [188]. However laboratory methods used to study this have relied on differentiating these trichomonads by their morphological appearance [334]. Though earlier investigators thought this form of species differentiation possible, [335] and recent epidemiological data in which TV has been found in women denying ever having sex [10] and also laboratory finding of *P.hominis* in the vagina [11] have lent some credence to the probability of this phenomenon, other studies have questioned the use of morphological and physiological criteria in trichomonad species identification [6].

In the present study using primers specific for vaginal, rectal and oral trichomonads in PCR assays on vaginal swab eluates from women in whom TV had been detected by morphology, no evidence was found for the involvement of human trichomonads other than TV in the aetiology of vaginal trichomoniasis.

The transient presence of *P.hominis* in the vagina is not improbable. As to whether this could establish infection making *P.hominis* a vaginal pathogen other than a mere "passenger" is not known. Stabler and Feo and Rakoff [335,336,337] showed in 1941 and 1942 that the intravaginal survival time of *P.hominis* was only rarely as long as 24 hours, and that in most cases all intestinal flagellates implanted in the vagina were dead within half an hour.

P.hominis, with its traditional site in the large intestines, could find its way into the vagina by being transferred via the perineum in situations of poor ano-genital

hygiene such as faecal staining or soiling of the perineum, cleaning away from the anus towards the vagina after defecation, or if vaginal intercourse follows a prior rectal penetration by a woman's spouse. It is probably one of these circumstances that led to the contamination of the vagina by *P.hominis* and its subsequent detection by PCR as reported by Crucitti *et al* [11]. Ano-genital and sexual behaviour data obtained from the Kumasi study participants indicated that these situations were uncommon and not associated with TV infection; 17% cases and 15% controls cleaned towards the vagina, and only 4% cases and 5.7% controls ever had rectal intercourse with their partners. The study did not ascertain whether or how often vaginal intercourse followed rectal intercourse. Genital examination of the women found no faecal staining of the perineum among any of the infected women and only 1 of 460 uninfected women.

There have been no published clinical reports of the finding of *T.tenax*, whose habitat is the mouth, in the vagina. Theoretically, during oro-vaginal contact, this could happen if *T.tenax* is present in the mouth. In studying this phenomenon, Stabler and Feo [338], inoculated human vagina with concentrated laboratory cultures of *T.tenax*. Whereas no permanent infections were established, *T.tenax* survived in the vagina for periods ranging 48-432 hours, with the majority surviving only up to 48 hours. Attempts at inoculating *T.tenax* directly from the mouth into human vagina however failed. None survived up to 48 hours.

This study looked at probable ways in which *T.tenax* could naturally be seeded into the vagina, including women receiving oral sex from their partners and also using their saliva to lubricate their vagina in situations of vagina dryness during sex. None of these practices were associated with TV infection.

In brief, with the use of molecular diagnostic techniques, no evidence for the involvement of trichomonads other than TV in the aetiology of vaginal trichomoniasis was found in this population of pregnant women. This probably re-echoes what was written by Feo and Stabler in 1942 [338] to wit "..more certainly than ever may we now say that the mouth, intestine and genital tracts of man are inhabited by 3 separate species of Trichomonads". They continue, "it might be suggested that as long as medical men refuse to accept this fact, just so long will the problem of the transmission of *Trichomonas vaginalis* remain in an unsatisfactory state". Unfortunately, what they did not appreciate then, and which is also the case now, is that a major problem with uncontrolled TV transmission is its asymptomatic presentation and the lack of a simple and affordable laboratory diagnostic which could be used in control programmes. Again and importantly, apparently unresolved issues such as the involvement of other trichomonads in the aetiology of TV infection warrant studying in the light of developments in science and technology, more so as a similar phenomenon occurs in veterinary practice.

8.2 Limitations to the study

The presence of *P.hominis* and *T.tenax* in this study was ascertained by PCR and not by morphology (eg microscopy and cultures). This was done to circumvent the problem of possible species misidentification with TV due to their similar morphologies. However, it would not be unusual that PCR assays could be negative, whilst morphological identification is positive, as was shown for TV in this study. This could have been noticed if parallel morphological identification was carried out. Possible factors that could predispose the study women to *P.hominis* and *T.tenax* colonisation of the vagina was largely absent in the pregnant women studied, as

discussed earlier. In a different population with poor ano-genital hygiene etc, results could be different.

9.0 RESULTS: PRESENCE OF TRICHOMONADS IN THE VAGINA, RECTUM AND MOUTH

The presence of the 3 trichomonad species in each of the 3 body sites i.e. vagina, rectum and mouth was investigated. From vaginal swab eluates, species specific PCRs for TV, *P.hominis* and *T.tenax* were done. From rectal swab eluates, PCR for *P.hominis*, *T.tenax* and TV was done, and from oral swab eluates, PCR for *T.tenax* and TV was done.

To establish the quality of material being processed in terms of adequacy of sample collection, the vaginal and oral swab eluates were checked for the presence of the human gene, β-2 microglobulin, and rectal swab eluates checked for the presence of *E.coli*, a commensal of the gut [277].

β-2 microglobulin was present in all the 633 oral swabs. β-2 microglobulin amplification was not possible in 12 of the 633 vaginal swabs after a 1:10 dilution. Thus only 621 vaginal swab eluates had trichomonads PCR assays done on them. *E.coli* was detected in all rectal swab eluates.

In this group of women, *P.hominis* was not detected in any site including the rectum. Also, *T.tenax* was not found in any site including the mouth. *T.vaginalis* was found in 161 of the 621 vaginal swab eluates only and none in the rectal or oral swab eluates. *P.hominis* and *T.tenax* were not found in vaginal swab eluates.

In summary, apart from the vagina, where *T.vaginalis* was found, no trichomonads were detected in any other site, even including their traditional sites.

9.1 DISCUSSION: PRESENCE OF TRICHOMONADS IN THE VAGINA, RECTUM AND MOUTH

Apart from the finding of *T.vaginalis* in its traditional site (vagina), not only was there no evidence of trichomonad cross contamination, *P.hominis* and *T.tenax* were also not detected in the rectum and mouth respectively, of women in the study.

P.hominis is acquired by faeco-oral transmission. Its prevalences have been reported between 0.1%-30% [339] in various population groups, and when present in the human gut, is regarded as a commensal [339]. Occasionally though, it has been found in diarrhoeic stools and its role in the disease process has been argued [340, 341]. It is not unusual that in this adult population *P.hominis* was not found, as most infections transmitted faeco-orally tend to predominate in children. Crucitti *et al*, using a similar assay also did not find any *P.hominis* in 112 adult stool samples submitted for routine parasitological and bacteriological analysis [11].

The negative results cannot be ascribed to poor DNA extraction procedures or inhibition in the PCR assay, as all the samples tested positive in the *E.coli* PCR assay which assessed the quality of sampling. However, in situations of low numbers of *P.hominis* in the host, sampling inadequacy cannot be ruled out.

Only one study has reported finding *P.hominis* in vaginal swab eluates [11]. Working on 3077 self collected vaginal swabs from women in Tanzania, Crucitti *et al* reported the finding by PCR assay of *P.hominis* in 2 samples. What this means in practice is not certain and the probability of peri-anal contamination of these non-health worker collected swabs cannot be ruled out. No *P.hominis* was found in the Kumasi study, where the vaginal swabs were nurse obtained after passage of a speculum.

The results of this study also discount speculations of the rectum being the source of *Trichomonas vaginalis* [10], from where the vagina is colonised.

T.tenax is found frequently in the mouth of persons with poor oral hygiene and those with advanced periodontal disease [170, 342]. The oral hygiene of study participants was apparently good and it is not unusual that the protozoon was not found in them. As with *P.hominis*, the integrity of the sampling process was ascertained by assaying the human cellular β-2 gene which was present in all the samples, thus ruling out inhibition of the PCR assay.

This study, like others [338] could also not find *T.tenax* naturally implanted in the vagina of women.

In conclusion, in this study, TV was found only in its traditional habitat. There was no finding of *P.hominis* and *T.tenax* either in or outside their traditional sites.

9.2 Limitations to the study

As in the previous chapter, factors that could predispose the study women to *P.hominis* and/or *T.tenax* colonisation, either in their traditional habitats or in situations of cross infection were largely absent. There was no evidence of faecal staining of the perineum of the women, they had good oral health, and the vast majority of the women did not receive oral sex from their partners. The situation could be different in another population group.

10.0 RESULTS: CLINICAL EFFICACY OF SINGLE DOSE METRONIDAZOLE THERAPY IN PREGNANT WOMEN

10.1 Metronidazole therapy

A total of 173 women positive for TV on wet mount microscopy and/or culture were treated with 2g single dose metronidazole. This was given to them in the clinic under the supervision of a nurse. They were asked to return to the clinic after a week for tests of cure.

10.1.1 Follow up rate

85% of the women (147) returned for their tests of cure as scheduled. The remaining 26 defaulters were followed up at the addresses given on enrolment. 21 women were traced to the addresses, but 5 could not be traced and were lost to follow up. Of the 5, 2 and 3 women were in the second and third trimesters of pregnancy respectively. 97% (168) of the women were thus available for tests of cure.

10.1.2 Symptomatology

3% (3/102) of the women complaining of vaginal discharge at enrolment still complained of the discharge at the test of cure. There were no new cases of vaginal discharge and no complaints of dysuria. 32 of 54 women still complained of genital itch. There were no complaints of genital ulceration even though previously 12% (21) of the women said they had a genital ulcer.

10.1.3 Sexual intercourse during treatment

All the women denied any sexual intercourse with their spouses during the week. This they did because of the counselling given before therapy. When asked what their partners' responses were, two-thirds of the women in the third trimester of pregnancy said they were "too pregnant" anyway and won't allow sex, and the others told their spouses they had been told so at the clinic in order to let the drug they had been given work properly and also not to endanger the pregnancy.

10.1.4 Sexual partner treatment

90% of the women (152/168) did not give/tell their partners of the prescriptions for metronidazole given them to be given to the partners, as this could lead to marital disharmony.

10.1.5 Drug adverse effects

Single dose metronidazole was well tolerated by 110 (65.5%) of the women. Side effects reported by the remaining women (58) included nausea (22.4 %), gastro-intestinal disturbances (36.2%) and metallic taste in the mouth (72.4%). One woman complained of dark urine. None of these was severe enough to send them back to hospital, or prevent activity. By the end of day 2, all the side effects had abated.

10.1.6 Cure rate

Of the 168 test of cure samples, TV was detected by wet prep and Inpouch culture in only 1 sample, giving a 99.4% cure rate. This woman denied any sexual intercourse during the intervening 1 week period. She was retreated with a further 2g single dose daily for 3 days, after which a test of cure was negative a week later.

10.2 DISCUSSION: CLINICAL EFFICACY OF SINGLE DOSE METRONIDAZOLE THERAPY IN PREGNANT WOMEN

Metronidazole has been used extensively worldwide for over 30 years, and continues to be a valuable antimicrobial agent in treating anaerobic obstetric and gynaecological infections and also protozoal infections. It is the drug of choice in many countries for the treatment of vaginal trichomoniasis and bacterial vaginosis. Even though there have been reports of resistance development to it [268, 286, 287, 288, 289, 290, 291], this has not been enough to warrant delisting it for such use.

A 99.4% cure rate at 1 week, defined as lack of positive TV cultures, was obtained in this group of women with the single 2g dose. Cure rates of 82-88% [114, 272, 273, 274] have been reported using this regimen. The 99.4% cure rate in this study could be the result of the directly observed manner metronidazole was given. Also, none of the women vomited the drug, as it was in general well tolerated. The women were also observed up to a week only. Re-infection from partners after prolonged periods of observation may have increased the failure rate. Thin *et al* reported a doubling of re-infection rates (1.4 to 3.8) between 7 days and 14 days observations [343].

One worry even with this high cure rate is the inability of the women to give their partners the free prescriptions for metronidazole. As most of the women were in monogamous relationships and either did not know whether their husbands were having sexual relations outside marriage or said emphatically that their husbands were not seeing other women, a prescription for a sexually transmitted agent could endanger marital harmony. The men could even be proven not to have TV infection after investigations, as again, the natural history of trichomoniasis in men suggests that it is of short duration and can be self-limiting [94]. However, if the men were the

sources of infection and still harboured the parasite, the result could be a vicious cycle of infection and treatment for the women.

Partner notification is an important aspect of STI control. Unfortunately in many male dominated societies which also happen to be societies where women are not empowered to make decisions on sexual matters including whether or not or when sex takes place, and where there is the possibilityof marital disharmony or even the woman being subjected to marital violence and thrown out of the house, partner notification is a taboo subject. Women find it increasingly difficult to tell their partners to attend clinics for investigation and treatment of STI. That is the situation in many traditional parts of Ghana, and this hampers STI control efforts. This is even made more difficult when the men, on realising they have any STI related symptomatology, attend chemist shops where they buy antimicrobials without the knowledge of their spouses. With a cure in sight, they argue with their spouses over them not being the sources of infection.

Even though adverse events were reported from 35% of the women, this was not severe enough to restrict its use. Hager *et al* also reported similar minor adverse events in his study population where 21% of patients receiving 2g metronidazole had side effects [114].

This is the first time that this 2g single dose regimen of metronidazole has been evaluated in Ghana. The excellent cure rate and tolerability warrant a consideration of its use for such indication in the country. This compared to the multi dose regimen minimises non-compliance, uses 62% less drug, and seems more practical for treatment of sexual partners who could be harbouring *T.vaginalis*.

Genital ulceration which had been complained of by 21 of the 168 at initial enrolment (although at clinical examination no ulcers were seen) seemed to have

disappeared even without specific treatment. In the Akan language, the word ulcer as used in the medical sense can translate into soreness (as in discomfort), burning pain or irritation. Though attempts were made during interviewing to convey the medical meaning, it is not improbable that the cultural and/or literal Akan meaning was assumed by the women. Indeed this difficulty has always been pointed out to clinical staff adopting the syndromic approach to management of STIs, emphasising the importance of genital examination.

This study has demonstrated the clinical efficacy of metronidazole therapy in Ghana for the treatment of vaginal trichomoniasis. The single case that was a failure responded to treatment with increased and prolonged metronidazole treatment, underscoring the fact that most situations of apparent metronidazole resistance in TV infection could be overcome with increased doses. However, for surveillance purposes a study of the minimum inhibitory concentration of metronidazole to this isolate would be important to assess the degree of in-vivo resistance. An attempt to do this failed as the isolate stored at -70° C could not be revived from its frozen state.

10.3 Limitations to the study

This was an observational study and did not compare any treatments. The clinical performance of this single dose regimen with the multi-dose regimen in Ghana could be better made in a comparison study of both regimens. However in studies from other countries there has been no significant difference in the clinical efficacies of both regimens.

Observing the 1 week treatment successes for longer, could have given an indication of whether the drug had been lethal or only inhibitory to the trichomonads. Also, the women were able to abstain from intercourse with their partners for a week, giving

various reasons why abstinence was necessary. The test of cure rates may have been different if the women were observed for longer as re-infections could have taken place.

11.0 CONCLUSIONS AND RECOMMENDATIONS

Prevalence data from various population groups underscore the fact that numerically, *Trichomonas vaginalis* infection has not been a minor STI, at least in the case of women. Estimates from the WHO confirm that it is the most common curable STI among sexually active women. Unfortunately unlike its counterpart sexually transmitted infections, gonorrhoea and chlamydia infection, important aspects of its epidemiology and clinical presentation have not been worked out. Again, even though an effective antimicrobial against it has been available for more than 30 years, which drug is still clinically efficacious today, many women are not treated, one reason being the absence of a good laboratory diagnostic test which can meet the needs of resource poor settings where infection prevalences are high. The absence of TV control programmes including screening for infection could be partly due to this lack of a good and feasible laboratory diagnostic test.

As well as being a common STI, trichomoniasis could also contribute to long lasting reproductive health morbidity for women and children born to infected women. Limited data is available suggesting a role for TV in the causation of prematurity and delivery of low birth weight infants. These same sequelae of TV infection have been used to argue for the importance of control of other treatable STIs. In recent times the role of TV in facilitating the acquisition of HIV has been recognised. It is probably time more effort was put into the control of TV.

This study has looked at some aspects of the epidemiology of TV infection in an urban population in Ghana, West Africa with a view to increasing our understanding of TV infection in order to help with its control.

In the group of pregnant women studied, various socio-demographic, behavioural and clinical parameters were found to be independently associated with infection. These included young age, being of neither Akan nor northern extraction, having no religious faith, douching and not using toilet roll for menstrual hygiene. Also, complaining of a vaginal discharge, clinically having an abnormal vaginal discharge, and a vaginal pH more than 5 were independently associated with infection. However, singly or in combination, these factors were not useful for the prediction of TV infection in this low TV prevalent antenatal population, their predictive values being less than the least sensitive laboratory diagnostic for TV currently used in many settings i.e. the wet prep.

Scoring systems and algorithms based on sociodemographic, historical and clinical parameters predicting sexually transmitted infections have been proposed for use in resource limited areas of the world where specific laboratory diagnosis of infection is either unavailable or too expensive. Though they may be useful, such systems have their limitations. They are population specific, useful only in those populations in which the factors were studied, as for example a risk factor for infection in a commercial sex worker population may not pertain among women in the general population. Also, some of these factors are static e.g ethnicity and religion, never changing for an individual through their life time, and thus wrongly profiling individuals.

Traditional laboratory diagnostic tests for TV, the wet prep examination and culture, and relatively new tests, enzyme immunoassay, latex agglutination, lateral flow dipstick, and PCR were compared for the detection of TV. The LAT with sensitivity and specificity of 91% and 99% respectively compared favourably with the most sensitive and specific test, culture (sensitivity 98.4%, specificity 100%) with a kappa

statistic of 0.93. In view of the many practical advantages of LAT over culture in terms of its use in a resource poor setting – same day test to allow for early treatment, ease of test training and performance, no other equipment needed, and relatively cheap, it has the potential for use in TV control programmes.

The LAT has the potential for use in public health programmes. First and foremost, the presently used syndromic approach for the management of vaginal discharges as used in many resource poor countries is useful in situations when women are symptomatic. However, adequate control of these infections needs the identification of infected women who are asymptomatic. In TV infection, as many as 50% infected women could be asymptomatic. Use of the LAT in screening programmes could identify such asymptomatic infections for cheap metronidazole therapy, complementing the syndromic approach. Secondly, even for symptomatic women with a vaginal discharge in low TV prevalent settings, the use of the syndromic approach can result in overtreatment of women. Incorporating the use of the LAT could augment and improve specificity for the management of TV infection.

In analysing a model of treatment interventions for trichomoniasis, Bowden and Garnett [12] indicate that identifying the infected (both symptomatic and asymptomatic) and giving appropriate treatment could be the most efficient means of controlling TV. Identification of TV infected subjects would be greatly augmented by a good screening tool that would detect most infections and allow prompt treatment. Currently available diagnostic tools do not allow for this. The LAT could fill this void. In high TV endemic areas which unfortunately are also often HIV prevalent settings, this simple and easy to use TV diagnostic would go a long way towards the control of HIV/AIDS and possibly reduce preterm, premature low birth weight deliveries.

This study also found no evidence implicating human trichomonads other than TV in the aetiology of vaginal trichomoniasis. It also confirmed the vagina as the sole origin of TV. Thus until there is evidence to the contrary, the addition of genital hygiene measures solely as a measure to contribute to TV control is unwarranted and could lead to anxiety.

In Ghana today, metronidazole still remains clinically efficacious in the treatment of vaginal trichomoniasis. Only low level resistance, which was adequately treated with increased doses of metronidazole, seems to exist. Surveillance of this situation though, is important.

In summary, demographic, behavioural and clinical factors have not been found useful in the prediction of TV infection. Also, the involvement of other human trichomonads apart from TV in the aetiology of vaginal trichomoniasis is questionable. We have in our hands a potentially good screening and diagnostic tool, the LAT for detecting *T.vaginalis* infection and there is already available a cheap single dose antimicrobial with cure rates over 80%. Is it not time routine screening and treatment programmes for TV evolved? This would not only lead to the reduction of adverse pregnancy outcomes in high TV endemic populations but could also be important in the control of HIV/AIDS in these countries.

1.1 Suggestions for further research

1. Even though this study found no evidence for the involvement of trichomonads other than *T.vaginalis* in the aetiology of vaginal trichomoniasis in this group of pregnant women, evidence from veterinary practice and some circumstantial evidence from man indicate the possibility of this phenomenon. Research in a different population group, especially in

the geographic area (East Africa) where such circumstantial evidence has emanated could shed more information on the subject.

2. That the latex agglutination test for the detection of *T.vaginalis* has the potential for use in resource poor settings is exciting. Evaluation of its use in other reproductive health settings, and also in tropical settings other than West Africa where environmental conditions are different e.g. higher temperatures would further inform on its stability and use.
3. Operational research incorporating the use of the LAT in the management of vaginal discharges may help inform its use.
4. The lateral flow dipstick could similarly have the advantages of the LAT for the detection of TV infections. Possible reasons have been given for its poor performance in this present evaluation. Further evaluations under appropriate conditions would inform its diagnostic value.
5. The evidence base for the epidemiologic treatment of male partners of women with trichomoniasis (using metronidazole) to prevent re-infection is not strong, as most of these come from observational studies. Not only are most TV infections in men asymptomatic, infections are also thought to be of short duration and self-limiting. Such men may therefore not be reservoirs of infection for subsequent transmission. A randomised controlled trial of metronidazole and placebo in partners of women treated for TV infection with an endpoint of infection status of the women 2-4 weeks after partner treatment, would inform this practice which is touted in all guidelines on the management of partners of women with TV infection.
6. The prevention of a STI within a stable relationship as in this group of mostly married women studied, is only as good as partners being notified,

investigated and treated similarly, otherwise the women find themselves in a vicious cycle of infection and treatment. It is worrying that in most situations in Africa, partner notification for STI prevention is less than adequate, due mostly to cultural factors. Also, women are not adequately empowered to make sure this happens. An in-depth anthropological and social science study into these and other reasons for poor partner notification and probable measures to deal with it would help greatly in STI control.

Appendix 1

FEMALE ENROLMENT QUESTIONNAIRE

ID	STUDY NUMBER		
	INTERVIEWER'S INITIALS		
FE101	INTERVIEW DATE	____/____/_____	
	ANC/OPD NUMBER		

How can we find you? (Positive landmarks)

2. I want to ask you some questions about yourself

FE201	How old are you?	|___|___| years	
FE202	Number of years of formal education	1= Nil; 2=Primary; 3= JSS; 4= SSS 5=Tertiary; 6=Other	
FE203	What is your main occupation?	1=student;2=trader 3=housewife;4=farmer; 5=professional	
FE204	What is your partner's occupation	1=student; 2=trader 3=farmer; 4=artisan 5=professional; 6=driver 7=unemployed 8=other……	
____	_____	_____	_____
FE205	Religion	1=Nil; 2=Christian; 3=Moslem 4=African traditional; 5=other	
FE206	Ethnic origin	1=Akan; 2=Ga; 3= Ewe; 4=Northerner; 5=other specify ……	

3. I would like to ask you some questions about your health and previous pregnancies

FE301	How many times have you been pregnant including this one?	|____| If answer is 1 →	→ FE307
FE302	How many of your children are alive?	|___|	
FE303	Have you ever had a miscarriage?	1=yes; 2=no →	→ FE305
FE304	If yes, how many	|____|	
FE305	Have you ever had a still birth?	1=yes; 2=no →	→ FE307
FE306	If yes, how many?	|___|	
FE307	How many weeks pregnant are you? (check ANC card to help work this out)	1=first trimester; 2=second trimester; 3=third trimester	
FE308	Do you currently have a vaginal discharge?	1=Yes; 2=No	
FE309	Have you been treated for a vaginal discharge before	1=Yes; 2=No →	→ FE312
FE310	If yes, when was this	1=within the past 3 months 2= within the past 6 months 3=more than 6 months ago	

FE311	How were you treated	1=tablets/capsules 2=local application (herbs) 3=pessaries/suppositories 4=douching 5=other……	
FE312	Do you currently have pain passing urine?	1=Yes; 2=No	
FE313	Do you currently have genital itching?	1=Yes; 2=No	
FE314	Do you currently have genital ulcer/vesicles?	1=Yes; 2=No	
FE315	Have you ever been treated for a genital ulcer	1=Yes 2=No →	FE318
FE316	If yes when was this	1= in the last 3 months 2=in the last 6 months 3=more than 6 months ago	
FE317	How were you treated?	1=tablets/capsules 2=local application (herbs) 3=pessaries/suppositories 4=douching 5=Other……	

FE318	Has your partner ever been treated for a sexually transmitted infection	1=Yes; 2=No 3=don't know	→ FE322
FE319	Was it for a urethral discharge?	1=yes; 2=no	
FE320	Was it for a genital ulcer/vesicles ?	1=yes; 2=no	
FE321	What was it?	1=Don't know 2=Other…… 3= 88	"88", if not applicable
FE322	What drugs have you taken in the past 2 weeks? (help patient to identify drugs)	1=routine antenatal drugs (ie folic acid, Iron, multivite, Bco); 2=analgesics; 3=antibiotics; 4=others (specify……)	

4. Now I want to ask to ask some questions about marriage

FE401	What is your marital status?	1=single; →	FE501 2=married monogamously; 3=married polygamously; 4=separated;5=divorced
FE402	How old were you when you first married?	|_____| years	*"88" if not applicable*

5. I would like to ask some questions about douching and menstruation

FE501	Have you ever douched?	1=Yes; 2=No; ⟶ 3=don't know	FE505
FE502	What do you use to douche mainly?(don't prompt)	1=plainwater;2=saline water; 3=soap; 4=dettol;5=shampoo; 6=herbs 7=other (specify….	
FE503	Why do you douche(do not prompt)	1=to feel clean generally; 2=to feel clean after menstruation; 3=to feel clean after sex; 4=to clear a vaginal discharge; 5=to prevent pregnancy; 6=other(specify ……	
FE504	Have you douched today	1=yes: 2=no	
FE505	What do you use when you menstruate?(main method)	1=sanitary pad; 2=tampon; 3=cotton wool; 4=folded cloth 5=toilet roll 6=other (specify……	

FE506	How do you use it?	1=insert in vagina; 2=line underwear; 3=both; 4=other	
FE507	On average how often in the day do you change it	1=never; 2=once; 3=twice; 4=>twice	
FE508	What do you use to clean after visiting the toilet?	1=toilet roll; 2=newspaper; 3=any paper(not toilet roll); 4=hand and water; 5=other (specify ……..)	
FE509	Which way do you clean after defecating?	1=front to back; 2=back to front; 3=either way; 4=other (specify…… ……..)	
FE510	Which way do you wipe after urinating?	1=don't wipe; 2=front to back; 3=back to front; 4=either way;	

6. Finally I would like to ask you questions about sexual intercourse. You are assured that all you say is confidential and will not be disclosed to any other person

FE601	Have you been circumcised?	1=Yes; 2=No → 3=don't know	604

FE602	At what age were you circumcised?	|____| years	
FE603	Why were you circumcised?	1=don't know 2=other (specify	
FE604	How old were you when you first had sexual intercourse?	|____| years	
FE605	When did you last have sex	1= 1-6 days ago 2=last week; 3=two weeks ago; 4=three weeks ago 5=last month 6= more than a month	
FE606	Do you think your partner could be having sex with somebody else apart from you	1=yes 2=no 3= don't know	
FE607	Do you or your partner use saliva on genitals when sometimes your vagina is dry before sex?	1=yes 2=no	
FE608	Does your partner sometimes give you oral sex?	1=yes 2=no	
FE609	Have you ever had anal sex?	1=never → End of questionnaire. 2=sometimes; 3=frequently	
FE610	When was the last time you had anal sex	1= This week; 2=this month; 3=more than a month	

FE611	Did your partner use a condom on this occasion?	1=Yes; 2=No	

Thank you for your participation in this study. Your responses are confidential.
Let participant see Study doctor

Appointment date for next visit (next week)

Appendix 2

FEMALE CLINICAL EXAMINATION

(Please explain procedure to study subject)

ID	Study number		
	Examiner's initials	|___|___|___|	
FC101	Date of Examination	|___|___| / |___|___| / |___|___| **D** **M** **Y**	

FC201	Is patient circumcised?	1=Yes; 2=No → FC203 3=can't tell	
FC202	Circumcision type	1= type 1; 2= type 2; 3= type 3 4=type 4 5=can't tell	
FC203	Abnormal vaginal discharge present?	1= yes; 2= no → FC208	
FC204	Does discharge look like yeast? (clumpy, cheesy)	1=yes 2=no	
FC205	Discharge Odour	1=normal 2=malodour	
FC206	Amount of discharge	1=normal 2=scanty 3=profuse	
FC207	Colour of discharge	1=clear 2=white/grey 3=bloody 4=green/yellow	
FC208	pH of discharge	|____|	

FC209	Amine test	1=positive 2=negative	
FC210	Vulval redness	1=Yes 2=No	
FC211	Strawberry cervix	1=yes 2=no	
FC212	Is perianal region stained with faeces	1=yes 2=no	
FC213	Ano-vaginal distance	|____| cm	
FC214	Treatment given	1=metronidazole 2=nil 3=other	

Swabs taken and labelled: |___| *HVS x 4* |___| *Rectal swab*

|___| *Oral swab*

Appointment date for follow up visit (within 7 days) ____/_____/_____

Appendix 3

FEMALE LABORATORY FORM

ID	Study number		
	Technician's initials		
FLS101	Date samples brought in	|___|__|/|__|__|/|__|__| D M Y	

Swabs received |__| HVS x 4 |___| Rectal swab |___| Oral swab

Results

FLS201	TV latex	1=positive; 2=negative	
FLS202	Wet prep	1=positive; 2=negative	
FLS203	InPouch TV	1=positive; 2=negative	
FLS204	If positive, on day	|____|	
FLS205	Candida	1=positive; 2=negative	
FLS206	Nugent score	|____|	
FLS207	BV (Nugent)	1=positive; 2=negative	
FLS208	BV (Amsel)	1=positive; 2=negative	

Swabs labelled and frozen |__| vagina swab PCR |__| rectal swab PCR
|___| Oral swab PCR |__| InPouch positive sample|___| InPouch envelope

Appendix 4

FEMALE FINAL VISIT

ID	STUDY NUMBER		
	INTERVIEWER'S INITIALS		
FFV101	INTERVIEW DATE	|____|/|____|/|____|	
	ANC/OPD NUMBER		
FFV201	How old are you?	|____|____| years	
FFV202	When were you last in the clinic?	1=one week; 2=one month; 3=other	
FFV203	Do you currently have a vaginal discharge?	1=yes 2=no	
FF204	Do you currently have genital itching?	1=yes 2=no	
FFV205	Do you currently have pain passing urine?	1=yes; 2=no	
FFV206	Do you have genital ulcer/vesicles?	1=yes; 2=no	

FFV207	How many times have you had sexual intercourse since we last saw you? (don't prompt)	1=nil; ⟶ 2=once 3= twice 4=thrice 5=more than 3 times	FFV210
FFV208	Did you use a condom?	1=yes; 2=no	
FFV209	When did you last have sexual intercourse?	1==today 2=yesterday 3=two days ago 4=three days ago 5=four days ago 6=five days ago 7=more than 5 days ago	
FFV210	What was your partner's response ?		
FFV211	Did you have any problems when you took the medicine we gave you?	1=no; 2=nausea; 3=vomiting; 4=dizziness; 5=headache 6=metallic taste in mouth 7=other (specify……	
FFV212	Did your partner take his medication ?	1=yes 2=no	
FFV213	If no, reason		

We will need to check if the infection for which you were given medication has cleared. The nurse will give you swabs to take the specimens

|___| SAVS x 1

Appendix 5

FEMALE LABORATORY (Final Visit)

ID	Study number		
	Technician's initials		
FLF101	Date samples brought in	|____|__|/|___|__|/|___|__| D M Y	

Swabs received |__| HVS x 1

Results

FLF201	InPouch TV	1=Positive 2=Negative	
FLF202	If positive, on day	|____|	

Samples labelled and frozen

Date frozen:

|__| InPouch positive sample

|__| InPouch envelope

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PUBLICATIONS

DIAGNOSTICS

Comparison of latex agglutination, wet preparation, and culture for the detection of *Trichomonas vaginalis*

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Objectives: To compare the performance of three diagnostic methods for *Trichomonas vaginalis* infection—latex agglutination, saline wet mount, and culture.

Methods: Vaginal swabs from 3807 women attending antenatal clinics were tested for the presence of *T vaginalis* by latex agglutination. All positives and the following two negatives were tested by wet preparation and culture.

Results: The prevalence of infection by latex agglutination was 5.4%. Using an expanded gold standard based on the wet mount and culture results, the sensitivity of the latex agglutination test was 98.8% (95% CI 95.9 to 99.9) and specificity was 92.1 (89.2 to 94.5). The kappa index for test agreement was 0.93 for latex and culture and 0.88 for latex and wet preparation.

Conclusion: The latex agglutination test is a highly sensitive test for detecting *T vaginalis* infection. It is a simple rapid test and has the potential for use in screening and diagnostic settings.

Trichomoniasis is a sexually transmitted infection caused by the protozoon parasite, *Trichomonas vaginalis*. It is the commonest curable sexually transmitted infection; the World Health Organization estimates that 170 million new infections occur each year.[1] The highest prevalence of infection has been reported from resource constrained countries[2–4] and in disadvantaged populations in developed countries.[5] Infection with *T vaginalis* may facilitate the transmission of HIV[6 7] and is associated with adverse pregnancy and perinatal outcomes.[5] Thus, the control of *T vaginalis* infection is important in reproductive health settings and also in the control of HIV/AIDS.

Diagnosis of *T vaginalis* infection in most parts of the world is carried out by the saline wet preparation ("wet prep") method, a technique which has not changed since it was first reported by Donné in 1836.[8] However, this technique has a low sensitivity of 30–80%[9–11] and requires trained and experienced microscopists. The gold standard in *T vaginalis* diagnosis—culture—has a higher sensitivity of 71–100%,[12] but requires an incubator with a constant electricity supply and relatively expensive culture media, in addition to an experienced microscopist. Further, it can take up to 7 days for results to be obtained, and currently is outside the reach of many health settings. In recent times, the sensitivity of *T vaginalis* diagnosis has been much improved by the use of nucleic acid amplification technology.[13 14] Non-invasive diagnostic material (self obtained swabs, tampons, and urine)[15 16] appealing to patients, have also been used. Polymerase chain reaction (PCR) currently, however, is also outside the reach of many diagnostic centres in resource poor settings.

A less technologically demanding technique with good test performance characteristics is needed, especially in resource poor countries. We compared a latex agglutination test with culture and Donné's wet prep technique for the diagnosis of *T vaginalis* infection.

PATIENTS AND METHODS

A total of 3807 consecutive women attending antenatal clinics in Kumasi, Ghana, were screened for vaginal infections between September 2002 and May 2003. Following informed consent, self administered vaginal swabs were screened for *T vaginalis* infection using the Kalon TV latex agglutination test (Kalon Biological, Surrey, UK). In this test, vaginal swabs were eluted by agitation in phosphate buffered saline. One drop of this eluate was mixed with a drop of test latex on a reaction zone on a black glass slide, and the slide manually rocked continuously for 2 minutes. Agglutination of the test, but not control latex, indicated presence of *T vaginalis* antigen.

All study subjects testing positive for *T vaginalis* on latex agglutination testing, and the following two consecutive women testing negative had two further vaginal swabs taken. The triplet of tests was not matched according to symptom presentation. The swabs were obtained by a nurse from the posterior fornix, after speculum insertion and were tested for *T vaginalis* by wet prep and culture. In the wet prep technique, the swab was agitated in 0.9% saline and a drop of this was observed under wet mount microscopy at ×100 for the characteristic morphology and motility of *T vaginalis*. Any observed trichomonad was confirmed at ×400. Culture was undertaken using the *T vaginalis* InPouch system (Biomed Diagnostics, San José, CA, USA) as previously described.[17]

Latex agglutination and wet prep microscopy were done on site within 10 minutes of specimen collection. Inoculation of the InPouch was also done on site within 10 minutes of specimen collection and incubated at 37°C within an hour of specimen inoculation. All three tests were read independently and blind to the results of the other tests by different technicians.

Test sensitivity, specificity, and predictive values were compared. Kappa statistic for tests agreement between latex and wet prep, and latex and culture was also determined.

RESULTS

Of the 3807 pregnant women, 206 (5.4%) were positive for *T vaginalis* on screening with the latex agglutination kit; 618 women (206 latex positives, 412 latex negatives) were selected for wet prep and culture testing. At presentation, 343 women (55.5%) were symptomatic for vaginitis (either vaginal discharge or itch) and 275 (44.5%), asymptomatic.

Table 1 Diagnostic comparison of latex agglutination, wet prep, and culture for detection of *Trichomonas vaginalis*

T vaginalis infection (n = 618)	Diagnostic test		
	Latex agglutination	Wet prep	Culture
True positive*	171 (27.7%)	141 (22.8%)	170 (27.5%)
False positive	35 (5.7%)	0 (0%)	0 (0%)
False negative	2 (0.3%)	32 (5.2%)	3 (0.5%)
True negative†	410 (66.3%)	445 (72.0%)	445 (72.0%)

*Wet prep microscopy or InPouch culture positive.
†Both wet prep microscopy and Inpouch culture negative.

Significantly more symptomatic subjects were positive on testing ($\chi^2 = 10.3$, $p = 0.001$); 64.5% of the 206 subjects testing positive with the latex as against 51% of the 412 testing negative, were symptomatic for vaginitis.

All but one of the latex positive samples was positive on culture, and all but one sample positive on latex agglutination testing was positive on wet prep. These two latex negative samples had discrepant results for wet prep and culture testing. Three samples with flagellates having characteristic morphology and motility of *T vaginalis* in the direct smear did not grow in culture.

Using an expanded gold standard for the comparison, patients were considered to have *T vaginalis* infection when either wet prep microscopy or InPouch culture were positive. They were considered negative for infection when both wet prep microscopy and Inpouch culture were negative. Table 1 shows the diagnostic comparison and table 2 test performance.

The kappa index which measures agreement between tests was 0.93 (95% CI 0.91 to 0.94) for latex and culture, and 0.88 (95% CI 0.86 to 0.90) for latex and wet prep.

DISCUSSION

In this diagnostic comparison, the test performance of the wet prep and culture are in conformity with other published studies.[12] Sensitivity of the latex agglutination test compares favourably with culture and is superior to the wet prep. Moreover, the test is simple to perform, requires no equipment other than a glass slide and mixing stick and gives a result in less than 3 minutes. This study is the second evaluation of the latex agglutination test and the first to be conducted in Africa. Carney *et al*[18] in their evaluation of this kit in the United Kingdom in 1988 reported a sensitivity and specificity of 95.2% and 99.4% respectively using wet prep and culture as reference standards. In spite of these encouraging results, the test has not been widely used.

Recent reports suggest that *T vaginalis* enhances the transmission of HIV,[6] and that symptomatic *T vaginalis* infection increases the amount of HIV shed in semen.[19] Moreover treatment of *T vaginalis* infection significantly lowers the vaginal and seminal HIV viral load in dually infected subjects.[20 21] Given the high prevalence of *T vaginalis* infection, its control could have a significant impact on the HIV epidemic in Africa, and may reduce the incidence of adverse pregnancy outcome.[5]

Two ingredients important in the control of sexually transmitted infections are accurate diagnosis and prompt treatment. Properly done, this will reduce the reservoir of infection and thus reduce the incidence of infection. In many health settings worldwide, wet mount microscopy is the preferred option for prompt diagnosis of trichomoniasis. This method, though timely and enabling patients to receive same day treatment, has a poor sensitivity[9–11] and patients could remain infected and untreated. Culture, with a much better sensitivity,[12] does not allow same day treatment, and patients, especially if asymptomatic for infection may continue to transmit infection. In many developing countries where the cost of return to the health facility can be substantial, patients may not bother to return for their culture results, thus prolonging infection, leading to further transmission and the possibility of sequelae. Partner notification efforts would also be defeated. This latex agglutination kit allows for prompt laboratory diagnosis of infection and thus treatment. With a sensitivity of 98.8% and a kappa index of 0.93 it compares favourably with culture, and its use could be more cost effective than culture. It provides same day results (test takes 2 minutes), thus saving time and costs due to incubation. At a present cost of £1 ($1.56) per test it compares with other rapid tests made for reproductive health settings. In resource poor settings where the cost of purchasing microscopes for wet prep and culture examinations may be prohibitive, and where trained and skilled personnel are unavailable, this latex agglutination test can fulfil such need. We have trained secondary school graduates to perform the test correctly. The kit as presently manufactured contains everything that is needed and no further purchases are needed.

In analysing a model of treatment interventions for trichomoniasis, Bowden and Garnett[22] indicate that identifying individuals with both symptomatic and asymptomatic infection and giving appropriate treatment could be the most efficient method of controlling *T vaginalis* infection. This would be greatly augmented by a good screening tool that would detect most infections and allow prompt treatment. Currently available diagnostic tools do not allow this. The *T vaginalis* latex agglutination test could fill that void. In high *T vaginalis* endemic areas, which unfortunately are also often

Table 2 Performance of the three diagnostic tests and 95% confidence intervals

	Latex agglutination	Wet prep	Culture
Sensitivity (%)	98.8 (95.9 to 99.9)	81.5 (74.9 to 87.0)	98.2 (95.0 to 99.6)
Specificity (%)	92.1 (89.2 to 94.5)	100 (99.2 to 100)	100 (99.2 to 100)
Positive predictive value	83.0 (77.2 to 87.9)	100 (97.4 to 100)	100 (97.9 to 100)
Negative predictive value	99.5 (98.3 to 99.9)	93.3 (90.7 to 95.4)	99.5 (98.1 to 99.9)

Key messages

- Present diagnostic tests for *T vaginalis* in resource poor settings are either insensitive or too expensive
- The *T vaginalis* latex agglutination test is a 2 minute test and its performance compares favourably with culture, the current gold standard in *T vaginalis* diagnosis
- It is a simple to use kit, requires no equipment, and is suitable for developing country settings

high HIV prevalence settings, this simple and easy to use *T vaginalis* diagnostic would go a long way in the control of HIV/AIDS and possibly reduce preterm, premature low birth weight deliveries.

About 6% (35) of samples were latex positive but negative for the traditional tests. It would be important to establish whether these are false positives by latex agglutination or false negatives by the traditional tests. Recent studies using molecular methods for the diagnosis of *T vaginalis* infection have shown culture to be less than 100% sensitive. Wendel *et al* comparing the polymerase chain reaction (PCR) and culture, reported sensitivities of 84% and 78% respectively[13] using an expanded gold standard of wet prep, culture, and PCR. Also, in the series by Tabirizi *et al*, *T vaginalis* infection prevalences of 9% by traditional tests as against 15% by PCR in the same population was reported.[14] This could also be important in many areas of the world where women are less empowered because of dependence on their spouses. False positive results could lead to considerable adverse social and relationship effects during partner notification.

Owing to the high sensitivity exhibited by nucleic acid amplification tests,[2 13 14] the sensitivity of *T vaginalis* PCR could well surpass that of the latex agglutination test, and there could still be missed infections using the latex kit in resource poor centres where PCR is unlikely to be used for diagnosis.

One of the weaknesses of the WHO vaginal discharge syndromic management approach is its poor specificity.[23] This can be greatly improved by the availability of rapid tests for vaginal discharge diagnosis. The WHO has commissioned field trials for rapid tests for the diagnosis of gonorrhoea and chlamydial infection.[24] When the results of these trials are favourable, and the diagnostics affordable, together with a rapid *T vaginalis* diagnostic, vaginal discharge management would greatly improve. Even in very low *T vaginalis* prevalent populations in developing countries, the use of the *T vaginalis* latex could be useful in eliminating infection and for effective partner notification. At a present cost of £1, this could be cheaper than the cost of blind therapy with metronidazole, unwarranted drug adverse effects, and the probable adverse social and marital outcomes with partner notification.

We have in our hands a good screening and diagnostic tool for *T vaginalis*. There is already available a cheap single dose antimicrobial against *T vaginalis* with cure rates over 80%.[25] It is time to evolve routine screening and treatment for *T vaginalis* in reproductive health settings.

CONTRIBUTORS

YAS, HAW, and DM designed the study; BKO and KAD trained the nurse-midwives and supervised clinical aspects of the study; YAS trained and supervised laboratory aspects of the study; data analysis was done by YAS and HAW; the manuscript was drafted by YAS, HAW and DM and written by YAS; all authors have reviewed and approved the final version of the paper.

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(In Press, Sexually Transmitted Infections)

Sexually Transmitted Infections Screening Guidelines

Trichomonas vaginalis infection

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Trichomonas vaginalis is a sexually transmissible protozoal parasite. It is the commonest curable STI; WHO estimate that about 170 million new cases occur annually (1). It is a common cause of vaginal discharge in women, in whom it may also cause vulval irritation and inflammation, dysuria and inflammation of the exo-cervix. It has been associated with dysuria and urethral discharge in men; but asymptomatic infection also occurs in both sexes. *T. vaginalis* infection is associated with low socio-economic status, and is more prevalent in developing than in developed countries (2,3). Opinions vary concerning whether or not *T. vaginalis* can be transmitted by non-sexual contact (4,5). A morphologically similar organism, *Pentatrichomonas hominis*, is a commensal of the human large intestine, but conventional wisdom has it that this organism does not multiply in the human reproductive tract.

Recommended tests

Microscopy of a wet mount preparation is the most commonly used diagnostic test for *T. vaginalis* infection. Characteristic motile flagellated protozoa are readily seen. Wet mount microscopy is approximately 70% sensitive compared to culture in women, and significantly less sensitive in men (6,7,8). At present, culture techniques are still regarded as the most sensitive and specific; they provide the "gold standard" against which other methods are judged. *Level of evidence: III*

Culture media vary in efficiency but Diamond's TYM medium (9) (sometimes with minor modifications) is amongst the best (10,11). Most tubes will be positive within 48 h but should be kept for 7 to 10 days before being finally discarded. A very convenient, but expensive, way of culturing specimens is the InPouch® system which appears to be at least as sensitive as conventional tubed media (12,13). *Level of evidence: III.*

A latex agglutination test which detects *T. vaginalis* antigen was described some years ago. This rapid and simple bedside test, which does not require electricity or special equipment, has been reported to have sensitivities of 95% and 98.8 % and specificities of 99% and 92.1% compared to culture for the diagnosis of *T. vaginalis* infection in women (14,15). This diagnostic test is available in kit form (TVlatex; Kalon Biological Ltd, Ash Vale, GU12 5QJ, UK). *Level of evidence: III*

More recently, several protocols have been described for the detection of *T. vaginalis* DNA in clinical samples using the polymerase chain

reaction (PCR) (16,17,18,19). Some of these assays appear to be more sensitive than culture although, as with PCR assays for *Chlamydia trachomatis* infection when they were first introduced, it is not immediately apparent whether samples positive by PCR and negative by culture represent false negatives by culture, or false positives by PCR. No PCR assay for *T. vaginalis* is currently on the market in the UK. *Level of evidence: III*

Who should be tested?

Until recently *T. vaginalis* has not been considered an important pathogen since, unlike other STIs, it was not believed to cause serious sequelae. Screening of asymptomatic individuals for *T. vaginalis* infection is therefore not currently recommended. *Level of evidence: III.* However, its importance is now being reassessed in the light of recent evidence that it is associated with adverse pregnancy outcome, and facilitates the sexual transmission of HIV infection (20, 21, 22). Further research is needed to confirm these associations and to prove that the association is causal. Recent trials have found that treatment of TV infection in pregnancy does not improve pregnancy outcome, and may be harmful (23, 24, 25).

Women attending clinics with a complaint of vaginal discharge should be tested for *T. vaginalis* infection. *Level of evidence: III.* It is generally recommended that sexual partners of infected women should be treated epidemiologically (26, 27, 28, 29). *Level of evidence: 1b.* Testing of male partners could in theory lead to further contact tracing in those who test positive. *Level of evidence: IV*

Men with urethral symptoms which persist after infection with *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Mycoplasma genitalium* have been excluded or treated should be tested for *T. vaginalis* infection (30, 31). *Level of evidence: III.*

Test of cure is only recommended in those whose symptoms persist after treatment. *Level of evidence: IV.*

Recommended sites for testing

In women, a swab should be taken from the posterior fornix at the time of speculum examination. *Level of evidence: III.* Self-administered vaginal swabs have been used in many recent studies, and are likely to give equivalent results (32). *Level of evidence: III.* First catch urine specimens, with or without centrifugation, have also been tested in women, but the sensitivity is less than that achieved with vaginal swabs. *Level of evidence: III.*

In men, urethral swabs or first catch urine (FCU) samples are recommended. The sensitivity of FCU can be improved by testing the cell pellet after centrifugation. Sensitivity can be improved by testing both a swab and a FCU (33,34). *Level of evidence: III.* Swabs from the sub-preputial space may also be tested, but this method of specimen collection has not been well validated. *Level of evidence: IV.*

Factors which alter tests recommended or sites tested
Nil

Search strategy

A Pub Med search of the English language literature was conducted, using the key words *Trichomonas vaginalis* and trichomoniasis. Personal libraries and the abstracts of recent meetings of the International Society for STD Research were also scrutinised.

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